

THE USE OF THE DOG

IN

LONG-TERM EXPERIMENTAL STUDIES

Thesis for the award of the degree of
Doctor of Veterinary Medicine in the
University of Zurich, Switzerland.

A. N. Worden

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Huntingdon Research Centre, England

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presented by

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1 : GENERAL INTRODUCTION

The dog has served for many years as an experimental animal and for such other laboratory purposes as the production of vaccines or sera for use against canine diseases. There are allusions to the ad hoc use of the dog, even in antiquity, for assessing the safety or otherwise of food-stuffs, and the expression 'Try it on the dog' has become a colloquialism in the English language. The use of the species in physiological and pharmacological investigations has become traditional, and the classical studies of Pavlov on conditioned reflexes and other mechanisms involved dogs.

During recent decades the dog has been widely employed in nutritional and toxicological studies, especially those of a long-term nature in which groups of animals are given different diets, or receive varying doses or levels of a test material, over weeks or months, or even years. The author was greatly impressed when, as a young student, he was privileged to visit the colony of experimental dogs maintained by the late Drs. Laidlaw (afterwards Sir Patrick Laidlaw) and George Dunkin at the National Institute of Medical Research, London, for their studies on the prophylaxis and treatment of canine distemper. These animals were bred specially for the purpose, and their general adaptability to laboratory life and their relationships with those working with them were in happy contrast to the situation often seen

in, for example, some physiological laboratories at that time. Nearly 40 years later the situation in this respect has by no means completely changed, at least in some countries, but the scientific validity of using healthy, well-adjusted and documented animals is much more widely recognized. Fortunately this need is in harmony with the views of the more enlightened bodies concerned with animal welfare, e.g. the Universities Federation for Animal Welfare, and the author was fortunate in being asked to edit the first general textbook on the care and management of laboratory animals (Worden, 1947), the publication of which was sponsored by this body, and to assist with much-enlarged later editions (Worden & Lane-Petter, 1957; Lane-Petter, Worden, Hill, Paterson & Vevers, 1967).

Knowledge of the dog as an experimental animal has grown at a rapid rate, and many thousands of original scientific papers are based, or partly based, upon its use for nutritional, toxicological and other investigations. The majority of such investigations have related to basal physiological observations, or to predictive studies designed to assist man, but the resultant knowledge has been of great value also to canine medicine and canine pathology. The veterinarian, fortunately, has come to play a significant rôle in research, and in the production or preparation of animals for research. His diagnostic skills are required in order to define and interpret experimental findings, and to separate spontaneous conditions or infectious disease from the effects of the

material under test. Particularly in pathology, however, he has to harness his skills to a comparative approach. In modern long-term nutritional or toxicological studies it is the comparison between control and treated groups - usually a graded series of treated groups - that is of prime significance. At the Huntingdon Research Centre (HRC), which studies over 2,500 dogs a year, the pathologists include both medical and veterinary graduates, working together and assisted by science graduates and research assistants, all concerned with establishing the nature and comparative significance of their findings. In the dog, as yet, there have been relatively few - by comparison with rodents - life-time or near-life-time studies upon experimental dogs, although some are summarized in the compilation edited by Andersen (1970), to which reference is made later. There have been few opportunities, therefore, for the experimentalist using dogs to add to canine gerontology. With the occasional use of the dog for 7-year carcinogenicity investigations, and with the current demand for studies lasting 7 or more years upon progestational steroids, or upon progestogen-oestrogen combinations, this gap is likely to be filled, and interpretation will require skills based upon veterinary practice and veterinary pathology. Already problems have arisen due to the appearance in laboratory Beagles of mammary neoplasia, the spontaneous or induced nature of which has been a matter for debate.

The present thesis is based upon the studies undertaken by the author and his colleagues at HRC and elsewhere over some 30 years, together with information drawn upon others who have employed the dog extensively. The bulk of the thesis had been prepared when the textbook edited and in part written by Andersen (1970) became available, and the text has been revised - in particular the section dealing with the Beagle - to incorporate many additional points or reviews from this valuable new work. Full acknowledgement is made to this and other sources, and to the parts that have been played by the author's colleagues.

The author has previously contributed reviews upon laboratory dog husbandry (Worden, 1947), canine nutrition (Worden, 1960; 1965) and the use of the dog as an experimental animal for assessing the toxicity of drugs (Worden, 1968b), and of compounds of agricultural and veterinary importance (Worden, 1970). He has dealt also with the lineage and behavioural characteristics of the species in relation to abnormal behaviour (Worden, 1959). An important source book on the species as such is that of Fiennes & Fiennes (1969), while monographs or texts dealing with separate branches of study are referred to appropriately in the text that follows.

2 : THE BASIS FOR THE CHOICE OF SPECIES FOR LONG-TERM AND FOR PREDICTIVE EXPERIMENTATION

The choice and use of animals for drug safety evaluation and for chronic toxicity testing in general have already been reviewed by the present author (Worden, 1968b; 1970, pp.46-95), who agrees with the principles enunciated by his colleague (Noel, 1962), viz. 'Since the main principle of toxicity testing is that the drug should be given to the living animal before its trial in man, the animal of choice is one that reacts like man. Unfortunately, there is no such animal, and compromise is necessary. For all routine studies, the animal should be (a) freely available, (b) able to live in comfort, and, if necessary, to breed in the country in which the tests are undertaken, (c) easy to handle with comparative safety, and (d) able to remain healthy and active on diets which are broadly similar to those of man'. Somewhat similar criteria were employed by Woodard (1965), who emphasized the importance of standardization of species or strain, experience in terms of past use, and the presence or absence of physiological response systems similar to those of man.

Worden (1968b) pointed out that it was usual for chronic animal studies to be conducted on at least two species and that the maxim appears to have been 'when in doubt, use the

rat and the dog.' In the United States and in Great Britain, and indeed in several other countries, these two species have been employed far more than any others for chronic investigations. There has therefore been built up a volume of information on the rat and dog that has not yet been reproduced for other species. The monkey has come into increasing use during recent years, and we now employ it fairly extensively. The pig, although it has been claimed to have a number of advantages, is used much less often for long-term tests on drugs. One reason is that its bodyweight is relatively heavy, and it therefore requires a considerable quantity of the test compound, which may of course be in short supply during the experimental stages of development. We employ the mouse extensively for carcinogenicity testing but usually not otherwise for long-term drug studies, and the rabbit for teratological and other procedures, but only rarely for a long-term toxicity experiment. For long-term studies on pesticides and herbicides, but not yet for human drugs, we have sometimes used the fowl, which is of course routinely employed for short-term tests on organo-phosphorus compounds and on carbamates. We have, on occasion, used other species, including the cat, for chronic studies, our only objections to the use of these other species, or even of unusual or exotic ones, would be (a) lack of sufficient background information on such species under the particular conditions of this type of experiment, and (b) the question of supply.

For some years it has been advocated that long-term safety evaluation or predictive studies should be based on the early introduction of comparative metabolic studies, as indicated specifically for drugs by Goldenthal (1968). With the growing sophistication of metabolic studies, including the availability of synthesized metabolites, radio-labelled or otherwise, this approach may yet reap dividends, and the author is indebted to his colleagues in the Department of Metabolic Studies and Organic Synthesis at HRC, and to their collaborators in the Medical Unit of University College Hospital Medical School, London, for their efforts to work towards a solution of the difficult problems involved. Early comparative metabolic studies have thrown up many confusing issues. Thus Baker (1969) recorded one instance of a compound that was administered in radio-active form to several species, the results in terms of urinary excretion over a 24-hour period being compared with those of non-labelled material in man. The amounts of parent compound excreted ranged from only 1% in the rat to 26% in the monkey, with only a low level in the dog but a relatively high one in the mouse. Another interesting example is that provided by Knaak & Sullivan (1967), without on this occasion comparable or even unlabelled control data in man, who found a considerable difference between the rat and the dog in the way in which the two species metabolized 1-naphthyl N-methyl carbamate, or carbaryl. The dog, unlike

the rat, appeared to be unable to liberate 1-naphthol or to hydroxylate carbaryl, but it was found to be capable of conjugating naphthol and appeared to conjugate carbaryl directly.

Noel (1969) has emphasized that when attempting to establish the metabolic pathways in man and experimental animals, the quantitative aspects should not be forgotten, and has drawn attention to the geographical and racial factors that may affect the response of man himself. He lists the factors that should be investigated as absorption, qualitative changes, quantitative changes, tissue distribution and rates of excretion, but points out the difficulties usually encountered at present in making satisfactory tissue determinations in either man or animals. 'Too much reliance should not be placed on blood levels (even for new antibiotics where blood levels are frequently of importance) for the action of the antibiotic may depend upon its ability to penetrate into certain tissue sites. Qualitative estimations of urinary excretion products may indicate the presence of a metabolite not excreted in the urine of man and, if this metabolite circulates within the body, it may be considered unwise to use that species in the present state of our knowledge.' These and other difficulties will have to be overcome if, to quote Worden (1970), more than lip service is to be paid to the results of comparative metabolism. In the meantime the dog is the most widely used non-rodent species. Benitz (1969) analysed 134 studies, lasting one

month or longer, the results of which were published in Toxicology and Applied Pharmacology between 1959 and 1966 and found that 43.3% were carried out in rats, 38.1% in dogs and only 6.7% in monkeys. Bushby (1966) reported that 71.7% of all the laboratories to which a questionnaire had been submitted by the European Society for the Study of Drug Toxicity used rats and dogs.

3 : BREEDS AND TYPES OF DOG EMPLOYED FOR TOXICITY
STUDIES, FOR NUTRITIONAL RESEARCH AND FOR
OTHER TYPES OF EXPERIMENTAL INVESTIGATION

Worden, Noel & Jolly (1963) reviewed the characteristics of various breeds of dog employed for laboratory use. To their description has to be added information on other types that have been employed for specific purposes, together with data based upon extensive use of this species for nearly another decade.

3 : 1 Mongrels or Crossbreds

The mongrel dog is still widely used today for pharmacological or acute toxicity studies, and in many physiological laboratories, although its use for any of these purposes was banned from 1960 at HRC. Farber (1945) considered that the special breeding of dogs for research use was too expensive for most purposes, and while his view is probably correct the alternative is to purchase animals from a suitable source. At the time when Barnes & Denz (1954) prepared their review of the experimental methods used in determining chronic toxicity, such canine data as were available had been based upon mongrels. As they pointed out, factors such as differences between breeds, litters and sexes were completely submerged in the obvious heterogeneity of the mongrels used, and some of the simpler criteria of toxicity such as growth and fertility tests could not be applied to this species.

There are many problems associated with mongrels additional to those specified by Barnes & Denz, including latent virus or bacterial infections, endo- and ectoparasites and a variety of temperaments. 'Moreover, it is sometimes difficult to ensure that they may not have been obtained in nefarious or doubtful circumstances by the persons from whom they are procured' (Worden, 1970).

In the United States it has been possible in many cities, at least up to recent years, to purchase animals for research from the pound, and it has been a frequent compromise to employ these for short-term studies and to use pure-bred dogs, usually Beagles, for definitive chronic studies. The practice of using mongrels for short-term investigations was adopted for a limited time at HRC but was rapidly abandoned: in one instance an animal that had been purchased from a dealer was found to have already been subjected to a partial gastrectomy. Although mongrels or a miscellany of dogs are still used in some laboratories in different countries, the disadvantages outweigh the advantages even for acute studies (Noel, 1962), and the accepted practice today should be to employ more standardized or homogeneous material.

3 : 2 Cairn Terriers

The Cairn is one of several short-legged and rough-coated breeds of terrier that come from Scotland and which are obviously linked with one another. Hubbard (1948) felt it probable that the Cairn Terrier, once known as the 'Short-haired Skye Terrier' probably represents the type from which the West Highland White, the Scottish, the Skye and the Clydesdale Terriers have all been derived. This type of dog was originally bred solely for work purposes and Hubbard cites the fifteenth century work of Bishop Lesley of Ross who stated that it creeps into '... subterraneous burrows, routs out foxes, badgers, martens, and wild cats from their lurking places and dens'. The animal that today is exhibited and sold as a housedog has a compact body with straight back and well-sprung deep ribs, a naturally short tail carried erect, and a hard profuse coat of hairs, with dense undercoat. The full sized male weighs approximately 6.8 kg and stands about 25 cm at the shoulder, while the female is slightly shorter and, if not overweight, averages about 5.7 kg.

The Cairn has not been employed thus far at HRC for nutritional or toxicological studies, but it is featured in the work of McCay (1949), who employed a colony at Cornell University, Ithaca, N.Y., for his work on canine nutrition. It is not known to the author whether in fact it suffers from the same disadvantages as the closely-related Scottish Terrier referred to in 3:3.

Burns (1952) refers to the fact that the Cairn Terrier, like the Scottish Terrier, is reputedly resistant to canine distemper.

3 : 3 Scottish Terriers

The Scottish Terrier is derived from the same ancestral stock as the Cairn (see 3:2), and according to Hubbard (1948) it was in about 1879 that the 'genuine' Scottish Terrier, or Scotch Terrier as Vero Shaw referred to it, was proclaimed by its breeders as separate from the other types that had hitherto been exhibited under the same and somewhat all-embracing title. Another name popularly given to the breed is Aberdeen Terrier, due presumably to the fact that many animals were bred in that part of Scotland.

The Scottish Terrier is of about the same height as the Cairn but is more powerfully built and heavier, with a somewhat short and thick muscular neck, a long and powerful muzzle, and strong, level teeth. It is capable of being a highly intelligent and devoted companion (see Worden, 1959). Unfortunately, however, the effects of breeding for exhibition purposes appear to have had some adverse effects, and Freak (1962) has stated that the breed is highly susceptible to parturition abnormalities.

Freak's findings, which admittedly were based upon her experience in general veterinary practice and not with laboratory dogs, nevertheless provide a somewhat alarming indication of the way in which a breed can develop abnormalities that - without expert veterinary help - could prove

almost self-limiting. The area in her practice had breeding kennels, of comparable size to those producing Scottish Terriers, responsible only for Miniature and Toy Poodles. In this case, however, the comparison was somewhat startling: of 222 cases of dystocia encountered, 121 occurred in Scottish Terriers and 6 only in Miniature and Toy Poodles. An indication of the relative proportions of Scottish Terriers in the different categories of dystocia recorded by Freak is given in Table I. (In the discussion following Freak's paper, Miss Mary Dalby reported that, in a kennel of Border Terriers, there had been only 5 normal whelpings out of 33 in 12 bitches, over a period of 11 years).

Worden, Noel & Jolly (1963) referred to the fact that this breed had been employed at HRC on one occasion for a chronic (2-year) toxicity experiment and had been found to have many disadvantages: 'It has moderately soft and long hair which needs frequent grooming, a time-consuming factor. The animal seems relatively more liable to skin conditions than many breeds. It must be kept in individual kennels because it has a tendency to fight, and in general it is a noisy breed. Repeated examinations of the urine have shown that in the absence of serum changes, bile pigments and occasionally bile salts will occur in the males of this species, the females remaining relatively but not always completely free of such changes. Scottish terriers are

also most susceptible to parturition abnormalities (Freak, 1962). One advantage of this breed is its uniform rate of growth with an adult weight of about 12 kg.'

In the 2-year study referred to, eventually published by Worden (1969), data were obtained upon bodyweight, haematology and blood chemistry values of Scottish Terriers employed as control animals and upon those dosed at two levels with the chlorinated hydrocarbon, telodrin, and these are summarized in Tables II, III and IV. The values for the telodrin-treated animals have been included since there was no evidence of any effect from the test compound, except for a slight increase in alkaline phosphatase levels, and a marginal effect upon bodyweight, during the second year at the higher (0.1 mg/kg/day) level, nor was there any detectable effect on food consumption or on routine urinalysis, erythrocyte sedimentation rate, or differential white blood cell counts. The organ weights, as percentages of bodyweight, are summarized in Table V, again with the test as well as the control data included. Liver weight was significantly higher ($P < 0.05$) and kidney and lung weights were also higher ($P < 0.1$) in the animals receiving 0.1 mg telodrin/kg bodyweight/day, but there was no evidence otherwise of any deviation from the control values. There was, incidentally, no evidence of any histological change in any of the tissues examined, including the livers, kidneys and lungs in all groups. Although relatively limited in

terms of numbers of animals, therefore, the data presented may be cited - with the minor reservations noted - as normal for the strain of Scottish Terrier employed up to the age of 2 years.

Blamey & Brand (1939) described the occurrence of cystinuria in two Scottish Terriers, from one of which cystine calculi were recovered. Urine samples from this animal contained about 250 mg cystine/litre, and cystine sulphur accounted for about 20% of the total sulphur present. Although the second animal did not have any calculi, cystine crystals could be identified in its urine, and it excreted from 200 to 400 mg cystine/litre.

Despite the disadvantages of the Scottish Terrier encountered in long-term toxicity investigations, individual animals were employed successfully for nutritional and metabolic studies by Worden, Waterhouse & Partington (1954, 1955), Worden & Waterhouse (1955) and Worden, Waterhouse & Pulman (1956). The animals behaved in the same way as in other breeds in refusing to void urine when suddenly transferred to a relatively small metabolism cage: one individual who was 4 years old at the time did not pass any urine for 3 of the first 4 days in which he was caged, but voided excessively large volumes on days 5 and 6. Nevertheless, with adaptation, excellent results were obtained and there did not seem to be the same problems of fighting with other breeds as when groups of Scottish Terriers alone were maintained.

Clarke, Heron, Featherstonhaugh, Forgays & Hebb (1951) employed 6 Scottish Terriers for long-term isolation studies, confining them in partly opaque cages from the time of weaning for from 7 to 10 months. Contact with attendants was eliminated, and maintenance activity was carefully controlled. At the end of this time the dogs showed pervasive diffuse excitement and an approach-withdrawal conflict towards their attendants (Melzack, 1954; Thompson & Melzack, 1956) and soon after release 'whirling behaviour', i.e. a rapid jumplike turning within a small spatial area, was observed and continued for up to 2 years (Thompson & Heron, 1954; Thompson, Melzack & Scott, 1956).

Reference has been made in 3:2 to the report that Scottish Terriers, like Cairn Terriers, have a lowered susceptibility to fits during the first or highly febrile stage of canine distemper. There is, however, a condition sufficiently prevalent among individuals of the breed to be termed 'Scotch cramp', although reported in other breeds also (Klarenbeck, Koopmans & Winsser, 1942; Smythe, 1945), and taking the form of a recurrent tetany. It is more common in the male, is seldom seen under 6 months of age and Burns (1952) suggests that it may reflect parathyroid dysfunction.

TABLE I

NUMBERS OF SCOTTISH TERRIERS AMONG 222 CASES
OF DYSTOCIA RECORDED BY FREAK (1962)

<u>Causes of dystocia</u>	<u>Total cases</u>	<u>Cases in Scottish Terriers</u>
Relative foetal oversize	77	64
Absolute foetal oversize	15	4
Foetal monstrosity or gross abnormality	2	0
Malpresentation, other than posterior	12	9
Posterior presentation of first foetus	35	34
Abnormality of maternal soft structures	4	2
Accidental abnormality of maternal pelvis	1	0
Slackness of abdominal wall	3	0
Primary inertia	41	19
Secondary inertia	44	37
Nervous voluntary inhibition of labour	17	6
Slow initiation of labour (? hormonal)	1	1
Slow initiation of labour (due to subclinical eclampsia)	7	1
Abortion near to term of dead foetuses	2	2
Death of some foetuses prior to parturition	10	4
Coincident illness	1	0

TABLE II

SCOTTISH TERRIERS

BODY WEIGHTS (kg) AT 13-WEEK INTERVALS OF DOGS EXPOSED TO TELODRIN OVER 2 YEARS

Weeks dosed	Controls						0.025 mg/kg						0.1 mg/kg					
	1♂	8♂	19♂	2♀	14♀	24♀	5♂	7♂	18♂	10♀	12♀	15♀	13♂	17♂	23♂	6♀	25♀	26♀
0	7.83	6.10	7.68	6.38	5.88	2.50	6.78	7.63	8.80	6.01	6.60	6.65	8.17	6.50	3.38	5.88	3.68	—
13	9.93	8.83	9.78	8.09	6.90	6.75	9.28	10.50	9.58	7.82	8.49	7.41	9.30	7.31	6.70	7.55	5.26	—
26	9.93	9.50	10.28	8.90	8.25	9.08	9.93	11.35	10.15	8.93	9.10	7.88	9.70	7.80	8.70	7.50	6.55	5.13
39	10.90	9.50	10.46	8.73	7.85	9.03	10.13	11.71	10.23	8.88	9.20	7.95	9.33	7.75	9.00	7.78	6.35	6.85
52	11.11	9.57	10.91	8.57	8.34	9.14	10.01	12.21	10.52	10.09	9.80	7.98	9.25	7.16	9.53	—	6.27	7.83
65	11.07	9.36	10.43	7.99	7.83	9.13	9.27	11.24	9.65	8.74	9.67	7.69	9.26	7.34	9.45	—	6.34	7.14
78	11.24	9.42	10.39	8.32	7.49	8.70	9.39	11.35	9.89	8.39	9.37	7.45	9.65	7.21	10.12	—	6.55	7.18
91	11.11	9.42	9.97	8.23	7.57	9.00	9.19	11.53	9.67	8.35	9.46	7.68	9.33	7.25	9.83	—	6.35	7.22
104	10.87	9.79	10.67	8.44	7.89	8.79	9.61	11.52	10.48	8.91	9.35	7.63	9.62	7.49	10.91	—	6.80	7.38

TABLE III
SCOTTISH TERRIERS:

HEMATOLOGIC VALUES IN DOGS DOSED WITH TELODRIN (GROUP MEAN VALUES)

Level of telodrin (mg/kg/day)	Duration of dosing (months)	PCV (%)	Hb (g/100g)	RBC (10 ⁶ /cmm)	WBC (10 ³ /cmm)
Control	2	47	14.4	5.72	13.0
	6	50	14.0	6.70	13.4
	12	52	15.3	6.90	11.3
	18	51	15.4	5.15	11.4
	24	47	15.4	6.63	13.0
0.025	2	47	14.4	6.19	11.0
	6	47	14.1	6.15	11.0
	12	52	15.3	7.09	9.6
	18	53	15.6	5.22	9.4
	24	52	16.6	6.88	12.6
0.1	2	48	14.5	6.23	10.7
	6	44	13.7	5.90	13.9
	12	52	15.3	6.93	11.3
	18	49	15.4	5.15	11.4
	24	50	17.0	6.83	11.4

TABLE IV

SCOTTISH TERRIERS

BLOOD BIOCHEMICAL VALUES OF DOGS DOSED WITH TELODRIN (GROUP MEAN VALUES)

Level of telodrin (mg./kg. day)	Duration of dosing (months)	Urea (mg./100 ml)	Glucose (mg./100 ml)	Serum proteins (g/100 ml)				Alkaline phosphatase (KA units ^a)	SGPT (Karmen units)
				Total	Albumen	α_1	α_2		
Control	2	35	82	6.5	4.0	(-)	(-)	(-)	(-)
	6	25	61	6.8	4.0	(-)	(-)	(-)	(-)
	12	26	(-)	7.4	3.3	(-)	(-)	(-)	(-)
	18	22	57	5.9	2.4	0.4	0.5	1.4	0.8
	24	19	46	7.4	3.2	0.5	0.7	1.9	1.1
0.025	2	30	80	7.4	4.9	(-)	(-)	(-)	(-)
	6	22	65	6.5	3.9	(-)	(-)	(-)	(-)
	12	34	(-)	7.8	4.0	(-)	(-)	(-)	(-)
	18	21	60	5.8	2.9	0.4	0.6	1.3	0.6
	24	17	49	7.2	3.3	0.5	0.8	1.7	1.0
0.1	2	25	78	6.5	3.7	(-)	(-)	(-)	(-)
	6	26	61	7.1	4.0	(-)	(-)	(-)	(-)
	12	30	67	7.1	3.7	(-)	(-)	(-)	(-)
	18	23	62	5.7	2.7	0.4	0.6	1.4	0.7
	24	20	59	7.0	3.0	0.6	0.7	1.8	0.9

^a King-Armstrong units.

TABLE V

SCOTTISH TERRIERS

ORGAN WEIGHTS AS % OF BODY WEIGHT IN DOGS DOSED WITH TELODRIN FOR 2 YEARS
(GROUP MEAN VALUES)

Level of telodrin (mg/kg)	Sex	Body weight (g)	Brain	Heart	Lungs	Liver	Spleen	Pancreas	Kidney	Thyroid	Adrenal	Gonad
Control	♂	10,070	0.69	0.87	0.66	2.71	0.38	0.20	0.50	0.008	0.012	0.221
Control	♀	8,040	0.79	0.85	0.64	3.26	0.25	0.25	0.50	0.009	0.0016	0.011
0.025	♂	10,270	0.81	0.77	0.64	2.60	0.54	0.21	0.58	0.008	0.012	0.269
0.025	♀	8,060	0.83	0.88	0.64	3.02	0.29	0.23	0.51	0.011	0.017	0.011
0.1	♂	9,040	0.78	0.73	0.76	3.58	0.23	0.23	0.59	0.009	0.015	0.217
0.1	♀	6,840	0.88	0.87	0.70	3.72	0.37	0.26	0.59	0.009	0.017	0.018

Hubbard (1948) states that fairly small Spaniels, which were probably the ancestors of the Cocker Spaniel, existed in Britain about the beginning of the seventeenth century. At that time, he believes, the one title of Spaniel was given to all the diverse types then in use: later the larger dogs that 'sprang' the game into the air, or startled it, became known as Springers or Starters, while the smaller dogs employed for flushing woodcock became known as Cocking Spaniels or Woodcock Spaniels, hence the modern name of Cocker Spaniel, which had become well known by 1880.

Hubbard describes the characteristics of the Cocker Spaniel as follows:

'The skull is domed or convex across the top, clean-cut, showing a distinct stop. The eyes are full, hazel or brown in colour, appealingly awake in expression; the ears are set low, long with fine leather and well covered with long, straight and silky hair, but no curls or ringlets: muzzle well developed and rather square with level mouth.

'The neck is long, thick and muscular, with a slight arch and neatly set on to fine sloping shoulders; the chest is deep and well developed, neither barrelled nor too narrow; the back has gradually changed from the very long heavily muscled type to a short and cobby back which in relation to the length of the leg suggests a squarely built

dog; the loins are very slightly arched; the legs are straight, well boned and feathered with compact and well-padded feet; the tail is docked, set rather low and carried horizontally (in action the tail is continually in movement - hence the nickname of "Wagtail").'

For breed purposes, the adult shoulder height is given as approximately 39 to 42 cm. and bodyweight as approximately 11.5 to 12.5 kg for females and 12.5 to 13.5 kg for males.

Scott (1958), who summarized the earlier observations of his co-workers and himself, showed that the Cocker Spaniel was among breeds that, at 5 months of age, were much less shy than, e.g. the Basenji. This difference is inherited on Mendelian lines, there probably being 2 genetic factors involved. Similar differences were found to exist in respect of crouching, which appears to represent the original setting that has survived in Cocker Spaniels since there has not really been any selection against it. (Modern Setters are really Pointers).

Although Freak (1962) stated that the numbers were too few to justify valid comparisons, her records of 222 cases of dystocia among breeding bitches showed that the incidence in animals other than Scottish Terriers was highest among Cocker Spaniels. In an earlier communication (Freak, 1948) she referred to the frequent failure of females to develop early maternal instincts, hysterical and neurotic types appearing to be frightened of their offspring and refusing to 'mother' them.

Worden, Noel & Jolly (1963) referred to a colony of Cocker Spaniels that one of them (DWJ) had maintained for many years for studies in parasitology, for which purpose the breed appeared to be well suited. The animals had been found to mix well and to run in a communal exercising ground, when fighting was rare. They had proved easy to manage and to be remarkably free from disease, and despite the apparent predisposition of the long ears to otitis, this had not proved a problem in the colony concerned.

Worden, Noel, Wheldon & Mawdesley-Thomas (1972) - see also Worden, 1970 - employed 32 young pure-bred Cocker Spaniels for a two-year toxicity study on the organophosphorus compound, gusathion. There were three test groups receiving the compound at different levels in the diet and a single control group. Prior to the commencement of the experiment all 32 animals were sampled, at 4 and again at 5 months of age, for a variety of haematological and blood chemical investigations. Since the animals were receiving a standard diet of Spiller's P62 diet (see Appendix III), and were all housed in the conventional HRC quarters they were taken to be a representative sample of normal dogs of this breed and age, and the opportunity was taken to calculate, for each series, the grand mean of the 32 individual results, its standard error and the 95% confidence limits: the results are given in Table VI. The group mean values for the 8 animals that served as controls over the

subsequent 2-year experimental period are given in Tables VII and VIII. Comparable data for the circulating cholinesterase levels are given in Table IX, while the organ weights - expressed as mean values and as percentages of bodyweight - are given in Table X. Male and female growth rates, or rates of bodyweight gain of these same animals are depicted in Figs. 1 and 2. The voluntary food consumption of the 4 males and 4 females over a 2-year period may be seen from Table XI, which provides a summary of the food left (as g/week), from the set daily allowance of 200 g dry diet.

The histological findings in all 32 animals, including the 24 that were dosed with the organophosphate over a period of 2 years were - with the exception of a cholangitis present in one animal - essentially similar and would appear to be representative of the 'normal limits' of laboratory dogs. There was evidence throughout of a minimal chronic inflammatory reaction that could be considered as indigenous and did not bear any relationship to the administration of the test compound. In one low-dose animal, a female, there was some squamous metaplasia of some of the larger bronchi, as described by Hajdu & Rona (1965) in the Beagle. There was in occasional instances, in dosed and undosed animals, a minimum hepatic cellular infiltration with cells of the chronic inflammatory type. In several animals there was a

moderate degree of cystic hyperplasia of the columnar epithelium of the gall bladder. Very occasional flecks of basophilic material were seen in the renal medulla, and in a few animals there was a minimal inflammatory reaction. One animal showed evidence of Toxocara canis infestation. In one animal also there was a single focus of cellular infiltration in a rubro-spinal tract in the medulla. In the pituitary of several animals there was cyst formation considered to be an embryological cranio-pharyngeal remnant. Unilateral testicular atrophy was seen in 2 males, while in one female there was evidence of a minimal degree of endometrial cystic hyperplasia. Some enlargement of the adrenal zona reticularis was seen in 2 animals, a male and a female. Other incidental findings included minimal cellular infiltration of the prostate, occasionally associated with areas of cystic dilatation, a minimal degree of cellular infiltration of the submucosa of the oesophagus and a slight increase in the amount of connective tissue in the gastric mucosa.

One of the 32 Cocker Spaniels died during the course of the study, in fact some 10 weeks after the level of the organophosphate compound had been raised in an attempt to produce an effect, but there was nevertheless little to indicate that the pathological changes in this isolated example were a direct result of treatment (Worden, 1970; Worden, Noel, Wheldon & Mawdesley-Thomas, 1972). A week before death the animal became ataxic, had a watery discharge

from both eyes, an increased respiratory rate with slightly laboured respiration, myosis, occasional bouts of vomiting and frank jaundice. On post-mortem examination there was obvious loss of flesh with yellow discolouration of the mucous membranes and of the remaining fatty and alveolar tissue. There was gross distention of the gall bladder, the common bile duct was grossly distended, but no evidence of obstruction. Histologically the hepatic architecture showed evidence of a cellular filtration which was particularly marked in the periportal areas. Reduplication of the bile ducts was seen and the picture was compatible with cholangitis. With Oil Red O, many macrophages were seen around the bile ducts that contained fat.

An individual Cocker Spaniel, a female weighing 14.6 kg, was employed in the studies of Worden, Waterhouse & Partington (1954,1955) on the utilisation and excretion of vitamin B₁ by the dog, and on the response to test doses of vitamin B₁ hydrochloride, and differed in its responses from the animals of other breeds employed by its continued excretion of the test dose over a period of several days, there being an actual rise in excretion on the 4th day. This very unusual finding may or may not have had breed significance, but it would be unwise to draw conclusions from a single case.

Data on the brain weight and measurements of Cocker Spaniels are included in Table XXX, and there are several

comparative references to Cocker Spaniels in 3:6, while the behaviour of Basenji X Cocker Spaniel crosses is discussed in 3:9 (see also Scott & Fuller, 1965).

Phillips (1945) described the occurrence of overshot jaw or 'pig jaw', as he termed it, in a strain of Cocker Spaniels, and believed that it had resulted from a crossing of English with American specimens. Burns (1952) does not appear to feel that his conclusion, viz. that its irregular appearance is due to a recessive gene with multiple modifying factors, to be very instructive.

Phillips & Felton (1939) reported that umbilical hernia in the Cocker Spaniel (as in Collies and in Bull Terriers) was due to multiple recessive genes and was not associated with any other type of hernia nor linked with sex, colour or deafness.

The average measurements of adult Cocker Spaniel brains are included in Table XXX.

TABLE VI

MEAN AND RANGE OF HAEMATOLOGY AND BLOOD
CHEMISTRY VALUES FROM 32 YOUNG COCKER SPANIELS
PRIOR TO EXPERIMENTAL DOSING

Investigation	No. blood samples	Mean	Standard error	Range Mean $\pm$ 1.96SE	Units
PCV	32	38.0	3.15	31.8 - 44.1	%
	32	37.5	1.08	35.4 - 39.6	%
Hb	32	11.1	0.98	9.2 - 13.0	g/100ml
	32	11.4	1.27	8.9 - 13.9	g/100ml
RBC	32	4.71	0.40	3.92 - 5.49	$10^6/\text{cmm}$
	32	3.85	0.43	3.01 - 4.89	$10^6/\text{cmm}$
MCHC	32	29.3	1.18	27.0 - 31.6	%
	32	30.3	1.11	28.1 - 32.5	%
MCV	32	81	3.09	75 - 87	cu
	32	96	5.05	88 - 108	cu
WBC Total	32	20.4	4.74	11.1 - 29.7	$10^3/\text{cmm}$
	32	16.7	4.17	8.6 - 24.9	$10^3/\text{cmm}$
N	32	13.2	3.58	6.2 - 20.2	$10^3/\text{cmm}$
	32	12.5	3.80	5.0 - 19.9	$10^3/\text{cmm}$
L	32	6.6	2.05	2.6 - 10.6	$10^3/\text{cmm}$
	32	3.4	4.14	0 - 7.6	$10^3/\text{cmm}$
Urea	32	36.3	5.94	24.7 - 48.0	mg/100ml
	32	35.8	5.79	24.5 - 47.2	mg/100ml
Total Reducing Subs	32	107	7.75	91.7 - 122.0	mg/100ml
	32	105	6.30	92.5 - 117.2	mg/100ml
Proteins Total	32	5.4	0.80	3.9 - 7.1	g/100ml
	32	4.9	0.22	4.4 - 5.3	g/100ml
Albumin	32	2.6	0.41	1.8 - 3.4	g/100ml
	32	2.8	0.30	1.5 - 2.7	g/100ml
SAP	32	14.5	3.41	7.9 - 21.2	KA
	32	17.9	4.35	9.4 - 26.5	KA
SGPT	32	9.0	3.90	1.4 - 16.7	RF
	32	10.6	4.31	2.2 - 19.1	RF
ICD	32	20.9	3.72	13.6 - 28.2	B&B
	32	24.47	4.03	16.6 - 32.4	B&B
Cholinesterase Plasma	160	0.69	0.075	0.54 - 0.84	pH
	32	0.68	0.065	0.55 - 0.80	pH
RBC	160	0.15	0.042	0.06 - 0.23	pH
	32	0.20	0.033	0.13 - 0.26	pH

TABLE VII

COCKER SPANIELS: HAEMATOLOGICAL VALUES (GROUP MEANS) OF 8 NORMAL
CONTROL ANIMALS DRAWN FROM THOSE REPRESENTED IN TABLE VI AND
MAINTAINED FOR A FURTHER PERIOD OF 2 YEARS.

Age (weeks)	PCV (%)	Hb (g/100ml)	RBC ($\frac{10^6}{\text{cmm}}$)	MCHC (%)	MCV (cu)	WBC ($\frac{10^3}{\text{cmm}}$)	Neutro - phils ($\frac{10^3}{\text{cmm}}$)	Lympho - cytes ($\frac{10^3}{\text{cmm}}$)	Pro- thrombin index	Platelets ($\frac{10^3}{\text{cmm}}$)
28	43	12.9	4.82	29	90	18.2	11.2	6.1	101	-
34	45	15.4	5.40	34	84	19.1	13.2	4.9	100	
46	45	15.3	5.49	34	81	14.6	11.6	2.3	100	191
58	50	16.1	6.18	32	80	13.2	9.5	2.8	101	-
74	46	16.0	5.60	34	83	12.9	8.8	3.6	100	233
86	45	15.6	5.31	34	86	11.9	9.0	2.3	100	-
98	48	15.8	5.90	33	81	10.7	7.2	3.1	100	184
110	49	16.6	5.67	34	86	12.0	8.9	2.5	100	-
115	50	16.6	5.88	33	86	13.0	9.4	2.9	100	260
122	49	17.0	5.94	34	82	12.7	8.4	3.5	100	190

TABLE VIII

COCKER SPANIELS: BLOOD BIOCHEMICAL VALUES (GROUP MEANS) OF 8 NORMAL CONTROL ANIMALS DRAWN FROM THOSE REPRESENTED IN TABLE VI AND MAINTAINED FOR A FURTHER PERIOD OF 2 YEARS.

Age (weeks)	Urea (mg/ 100ml)	Glucose (mg/ 100ml)	Serum protein (g/100ml)			SAP (KA units)	SPGT (RF units)	KD (B & B units)
			Total	Albumin	Globulin $\alpha 1 \quad \alpha 2 \quad \beta \quad \gamma$			
28	35	93	5.9	2.7	0.6 0.6 1.4 0.7	11.8	11	24.0
34	43	95	6.4	3.1	0.6 0.6 1.4 0.8	11.9	10	24.4
46	38	92	6.8	3.1	0.5 0.5 1.6 1.1	9.8	14	24.5
58	45	99	7.0	3.4	0.5 0.6 1.6 0.9	4.3	26	20.4
74	35	102	5.6	2.6	0.4 0.6 1.2 0.8	7.2	24	24.4
86	40	92	6.1	2.7	0.4 0.5 1.5 1.0	7.1	24	8.7
98	33	90	6.4	3.0	0.6 0.6 1.3 0.8	6.6	27	15.3
110	32	99	6.3	3.2	0.5 0.6 1.4 0.6	6.3	22	14.7
115	38	100	5.9	3.0	0.4 0.6 1.3 0.6	7.6	19	13.6
122	32	92	6.4	3.1	0.6 0.6 1.4 0.7	6.4	22	13.7

TABLE IX

COCKER SPANIELS: CIRCULATING CHOLINESTERASE LEVELS
 (Δ pH UNITS, GROUP MEANS) OF 8 NORMAL CONTROL ANIMALS
 DRAWN FROM THOSE REPRESENTED IN TABLE VI : (1) BY
 ORIGINAL METHOD OF MICHEL (1949): (2) USING MODIFIED
 BUFFER AND SUBSTRATE CONCENTRATIONS OF WILLIAMS, FRAWLEY,
 FUYAT & BLAKE (1957)

<u>Age</u> <u>(weeks)</u>	<u>Plasma cholinesterase</u>		<u>RBC cholinesterase</u>	
	(1)	(2)	(1)	(2)
21	0.71	-	0.12	-
22	0.68	-	0.09	-
23	0.63	-	0.14	-
24	0.66	-	0.18	-
25	0.64	-	0.15	-
26	0.46	-	0.11	-
30	0.45	-	0.15	-
34	0.50	-	0.15	-
38	0.60	-	0.15	-
42	0.61	-	0.17	-
46	0.52	-	0.22	-
50	0.52	-	0.17	-
54	0.54	-	0.11	-
58	0.63	-	0.18	-
62	0.52	-	0.13	-
66	0.55	-	0.13	-
70	0.31	-	0.15	-

(Table IX - Continued)

<u>Age</u> <u>(weeks)</u>	<u>Plasma cholinesterase</u>		<u>RBC cholinesterase</u>	
	(1)	(2)	(1)	(2)
74	0.53	0.48	0.10	0.56
78	0.48	0.59	0.13	0.66
82	0.53	0.62	0.17	0.71
86	-	0.72	-	0.74
90	-	0.70	-	0.67
94	-	0.58	-	0.82
98	-	0.58	-	0.74
102	-	0.68	-	0.82
106	-	0.62	-	0.69
110	-	0.72	-	0.67
114	-	0.81	-	0.77
118	-	0.68	-	0.82
122	-	0.67	-	0.61
126	-	0.23	-	0.58

TABLE X

COCKER SPANIELS: MEAN ORGAN WEIGHTS (g) OF 8
 ANIMALS (4 male: 4 female) DRAWN FROM THOSE
 REPRESENTED IN TABLE VI AND EXAMINED AT 126
 WEEKS OF AGE (MEAN BODYWEIGHT 12.72 kg)

<u>Organ</u>	<u>Weight</u>	<u>Weight in % bodyweight</u>
Brain	77.3	0.61
Pituitary	0.07	0.58 ($\div 10^3$)
Heart	103.5	0.81
Lungs (2)	102.2	0.80
Liver	381.7	3.01
Spleen	80.8	0.64
Pancreas	28.1	0.22
Thymus	6.5	0.51 ($\div 10$)
Prostate	7.7	0.56 ($\div 10$)
Uterus	17.2	1.57 ($\div 10$)
Kidneys (2)	65.0	0.51
Thyroids (2)	1.09	0.86 ($\div 10^2$)
Adrenals (2)	1.50	0.12 ($\div 10$)
Testes (2)	27.4	0.20
Ovaries	1.30	0.01
Spinal cord	19.4	0.16

TABLE XI

MEAN FOOD RESIDUES OF 4 MALE AND 4 FEMALE
COCKER SPANIELS OFFERED 200 g DRY DIET/
DOG/DAY OVER A PERIOD OF 2 YEARS
(11,200 g/4 DOGS/WEEK)

<u>Age</u> (<u>weeks</u>)	<u>Food residue (g/4 dogs/week)</u>	
	<u>Males</u>	<u>Females</u>
22 - 58	401	2,302
59 - 79	72	2,364
80 - 106	41	3,171
107 - 126	110	2,923

FIGURE 1

Bodyweight gain of cocker spaniels

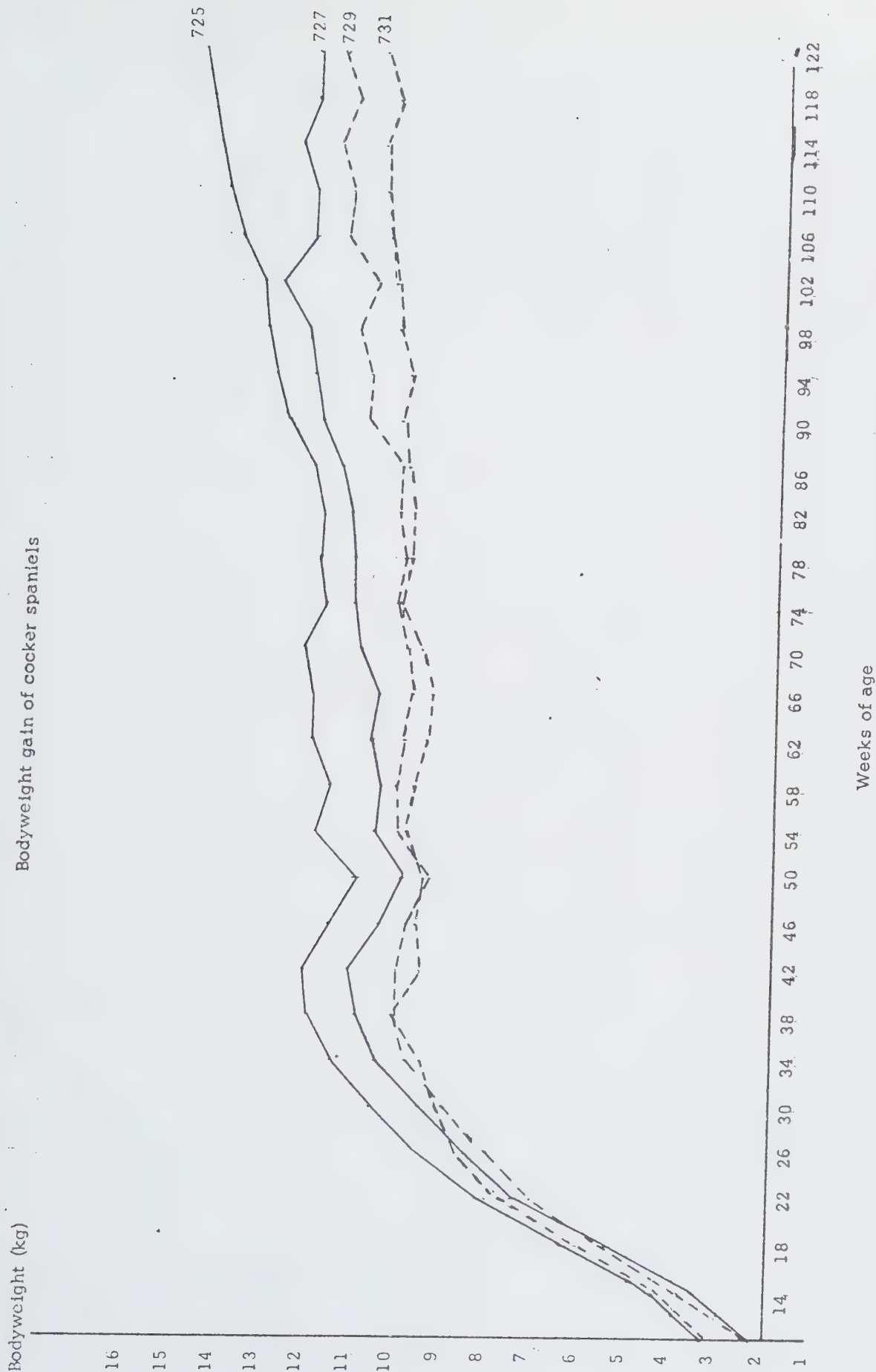
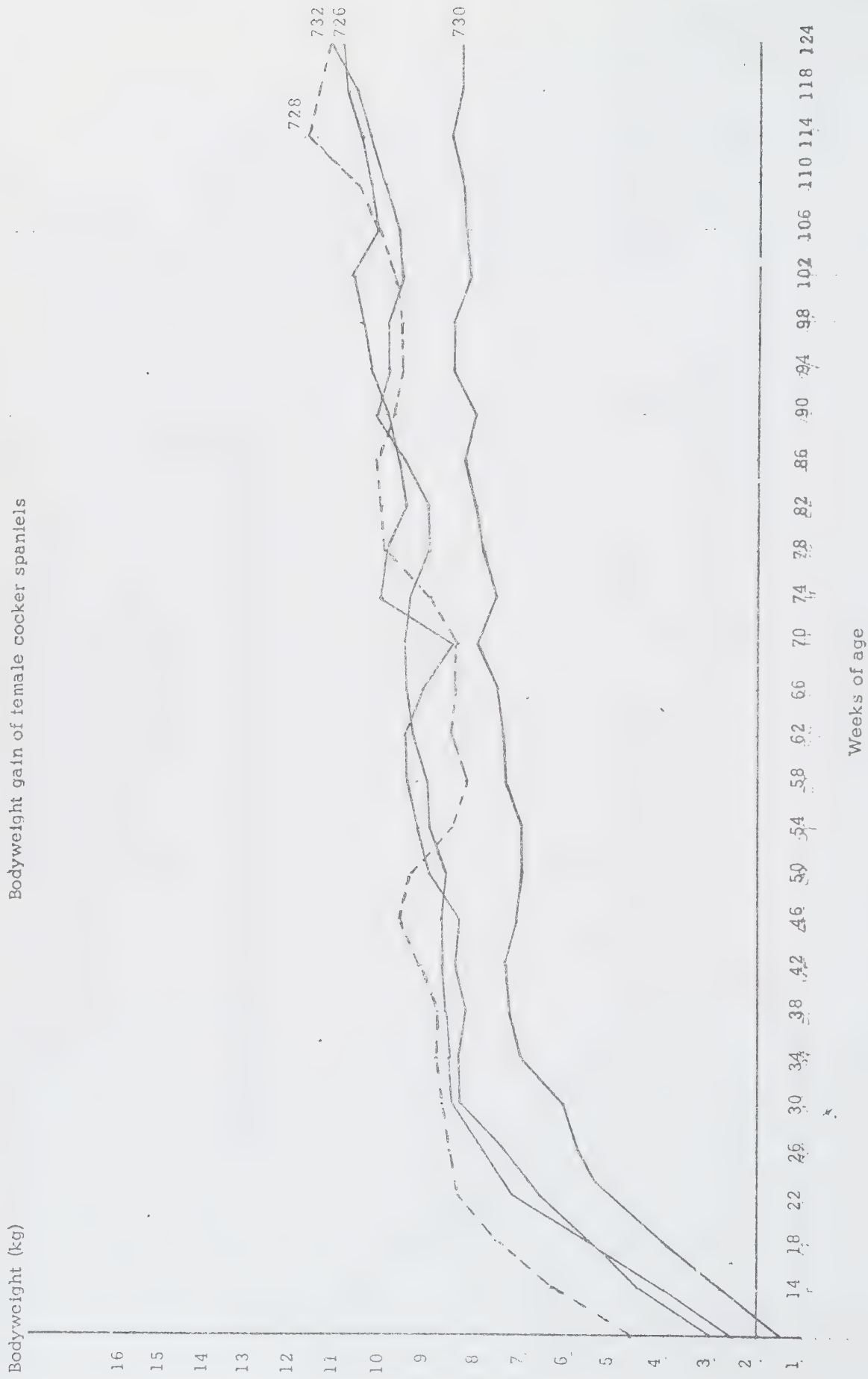


FIGURE 2

Bodyweight gain of female cocker spaniels



Hubbard (1948) states that although the two varieties of the Welsh Corgi differ in many respects they have a common origin and that, if one variety is the more ancient, it is probably the larger Cardiganshire type. 'The etymology of the breed name is from "cor" meaning "dwarf" and "ci" meaning "dog", but Spurrell's dictionary of the Welsh language (published 1859) gives the meaning as "cur dog". The former derivation is generally accepted but possibly because the term "cur" may have sounded displeasing to enthusiasts at some time or other. However, the name was not derogatory in any sense, for more than a thousand years ago references were made in Welsh Laws codified by King Hywel Dda to Curs as distinct from "Trackers, Greyhounds and Spaniels".... From the fifteenth to the end of the nineteenth century the Welsh drovers were renowned for the large herds of cattle which they brought to the Smithfield Market, London, across hundreds of miles of rude highway. Their black Welsh cattle were shod for the journey and subsisted on pastures adjacent to the route. The early type of Corgi was certainly employed by these drovers, who called them Ci Sawdl ("Heelers") as the dogs were trained to nip the heels of the stragglers of the herd. For many years also "Heelers" have been used in rounding up the hardy Welsh mountain pony in conjunction with local Sheepdogs.'

Hubbard regards the Vallhund or Västgöta Spitz, a small Swedish herding dog, as remarkably like the Corgi and is convinced that the two breeds are very closely related (see also Hubbard, 1947). He likens the head to that of the Spitz family, and in writing of the Pembrokeshire variety he states: 'The skull is wide between the ears with well-filled-out cheeks and only slight stop; the eyes are bright and alert, round and hazel; the ears are wide at the base, tapering to a rounded tip and carried erect and facing the front, open to catch the least sound as in the German Shepherd Dog (the ears are quite mobile); the muzzle is rather broad before the eyes and tapering to a rather finer point than in the Cardiganshire variety, with strong level teeth... The body is long but firm, a solid body of muscle. The back although laid over such short legs and being of good length does not sag, and shows a slight arch over the croup; the chest is fairly deep; the tail is either absent or short-docked to a mere stump (the common birth of absolutely tail-less Pembrokeshire Corgis is an indication of ancient lineage)... The coat is dense, short, and rather harsh in texture, but not wiry as in Terrier breeds (an approximation is to that of the German Shepherd Dog).'

For breed purposes, the shoulder height in either sex is not supposed to exceed 30 cm., while the bodyweight is generally about 10 kg for males and about 9 kg for females.

Worden, Noel & Jolly (1963) wrote of the Pembrokehire Corgi that: 'It is short-haired and needs little attention apart from the occasional brush and infrequent bath. It is a solitary animal and settles well in individual accommodation. It is extremely easy to handle and the old tale of its spiteful nature is, in our experience, untrue. Its growth rate is uniform with an adult weight of approximately 10 kg. It is easy to carry out routine experimental procedures with this breed; for example, if the animal becomes restless when a sample of blood is being withdrawn from the forelimb, it tends to give a sustained pull away, rather than a jerk which does not allow the operator to let the syringe ride with the leg movement.' It must be added, however, that since this was written there have been several instances of serious fighting, with some deaths, among Corgis that have escaped from their individual quarters at HRC.

For several years the Pembrokehire Corgi was the main breed of dog employed at HRC. Worden, Noel & Mawdesley-Thomas (1971) have described the use of the breed in a 2-year toxicity study on the organophosphate insecticide, ronnel, and a copy of their paper is appended to this thesis, and provides information comparable to that recorded in 3:4 for the Cocker Spaniel in relation to food consumption, bodyweight gain, haematology, blood chemistry, circulating cholinesterase and organ weights, together with data upon brain cholinesterase, bromsulphthalein clearance and bone marrow counts.

Noel (1969) has summarized electrocardiographic data on 378 Pembrokeshire Corgis, and on 986 Beagles, at HRC, and the results are given in Table XII, together with records of heart rates for these two breeds based on significant numbers of animals.

Freak (1948) suggested that in the Corgi, as in some other breeds (including Sealyham Terriers, Yorkshire Terriers and King Charles Spaniels), a wide range of size variation within the breed led to the production of some pups that were oversized relative to the size of their dam.

Cozens & Mawdesley-Thomas (1966) recorded reduplication of the pituitary in a male 6-months-old Corgi at HRC, and it was considered that the abnormality probably occurred early in foetal life, centrally rather than in the pouch of Rathke.

Clark & Cuddeford (1971) include 2 Corgis in their total of 13 male dogs of different breeds with cystine urolithiasis. The urinary cystine concentration of the 2 Corgis, viz. 65 and 80 mg/litre respectively, were below the mean of 180.8 ± 45.2 for the 13 cases, but well above the mean of the 13 control dogs of 40.2 ± 15.8 , which included a single Corgi with a value of 7 mg/litre. All affected animals had high urinary concentrations of threonine, serine and lysine.

TABLE XII
ELECTROCARDIOGRAPHIC DATA
AND HEART RATES IN BEAGLES AND IN
PEMBROKESHIRE CORGIS

Parameter	Age (months)	BEAGLES			CORGIS		
		No.	MEAN	(95% CL)	MEAN	(95% CL)	No.
P-R (seconds)	ALL ages	986	0.09	(.06 - .12)	0.09	(.06 - .12)	378
Q-T (seconds)	"	986	0.18	(.14 - .22)	0.18	(.13 - .22)	378
QTc	"	986	0.27	(.25 - .30)	0.27	(.22 - .32)	378
HEART RATE (beats/min)	̄4	474	156	(117 - 195)	153	(111 - 196)	158
	+1	424	148	(106 - 190)			
	+3	166	148	(103 - 181)	130	(90 - 170)	84
	+6	152	127	(76 - 177)	133	(94 - 172)	82
	+12	98	119	(69 - 167)	123	(90 - 156)	58

The Beagle, a small hound, is the most widely employed purebred dog in toxicological studies in the United States of America, England and elsewhere in Europe and has almost universal acceptance as the laboratory dog of choice for this type of investigation. Its original employment in the U.S.A. may have been due largely to the availability of sufficient numbers of suitable age, sex and litter of origin for adequate test and control groups to be mounted. Although it is believed that the ancestors of the Beagle came to Britain from France, the breed as such, with several of its varying types, was largely developed in the United Kingdom and exported to the United States (Hubbard, 1948). Many laboratory dogs on the Continent of Europe, and in other countries such as Japan, have been imported from the U.K. or the U.S.A.

Hubbard writes that: 'The Beagle is not unlike a small Harrier in appearance, and is similarly built for hare-hunting. The breed is apparently descended from the old Southern Hound (an offshoot of the Talbot Hound brought over by William the Conqueror), and one sometimes comes across a specimen exhibiting several characteristics of this low-to-ground type; the Basset-like type is not in favour, however... Beagles vary in size, type and coat. The Kerry Beagle is quite the highest on the leg, being fully as much as 22-23

inches in height, and not well known outside Eire..... On the other end of the scale the Pocket Beagle (or Rabbit Beagle) stands only 10-18 inches, and represents a rabbit-hunting dog.... The Welsh Beagle is in a class of its own, being rough-coated and standing about 15-18 inches, the smaller dogs being used to hunt the common brown hare (Lepus europaeus) and the taller dogs employed in hunting (in North Wales) the blue and Irish hares (L. timidus and L. hibernicus) found in the mountains..... The most popular type is the Smooth-coated Beagle, standing about 14 inches at the shoulder, and this is that which is generally exhibited... In the United States Beagles are popular.... American stock mostly having been imported from Britain..... The head is moderate in length and width, showing a slight dome and a pronounced peak, with a definite stop; the eyes are dark hazel or brown; the ears are set low, long and pendant like folds of velvet; the muzzle is of fair length without being snipy... The body is muscular and fairly deep... The back is straight and shorter than that of the Basset Hound; the chest is deep and roomy; the legs are strong and muscular, straight and well boned, with round rather compact feet; the stern is set high, thick and fairly long.... The coat is short, close-lying and smooth in texture.'

The reference to the Kerry Beagle is interesting. Whatever its origin, and presumably the strain must at some time have been crossed with a larger-legged hound, or have

developed phenotypic manifestations from an ancestral component, the excellent Beagle bred and maintained by the Hoechst Laboratories in Germany is of about this height and length of leg.

Douglas and Carol Appleton (1970), who supply a very high proportion of the Beagles employed for research in England and in other countries in Europe, have produced a work that gives an authoritative account of the breed and its uses, with a chapter on the Beagle in research to which the author's colleague, Dr. Ralph Heywood, has contributed. They refer to the first mention of Beagles or 'kenettys' in 1475, to the fact that the Kerry Beagle is the result of crossing with Harriers or Foxhounds, and to the first exports to the United States in 1884.

Andersen (1970) acted as editor of a work on the Beagle as an experimental dog, prepared under the auspices of the United States Atomic Energy Commission, which is a mine of information compiled by many authors on the husbandry, anatomy, physiology, pathology and special uses of the breed. This work became available after the present thesis had been planned, and the opportunity has been taken to incorporate data that supplement those from the records at HRC. Andersen himself, in the introductory chapter, quotes from a survey of 18 large commercial and institutional Beagle colonies in the U.S.A. the size preferred for experimental use in that country as follows:

	<u>Adult male</u>	<u>Adult female</u>
Bodyweight (kg)	10.5	9.9
Height at withers (Cm)	34.2	33.0
Torso length (cm)	52.5	50.1

Whitney (1955) described the specific lines of Beagles available in the U.S.A., while Ginsberg & Slatis (1962) reported on the results of a survey of 450 American Kennel Club litter registrations, from which it appeared that about 10% of Beagles fell into the inbred category.

Andersen refers to attempts that have been made to produce hybrids of Beagles with other breeds, e.g. with the Cairn Terrier to yield the 'Keagle' and with the Basenji. The Beagle-Basenji cross is stated to have been midway in size between the two breeds, to have had Beagle conformation except for wrinkling of the skin about the head and to have been easy to handle but not barkless, although with a quieter bark than that of the Beagle itself. He refers also to the attempts made in his own kennels to raise Beagles in a large pen adjacent to a well-travelled road, so as to permit of more human contact, and states that this reduced the 'critical distance', i.e. the distance at which a given animal will respond in some manner to the approach of man or of some other animal (Hall, 1965), adding that Beagle pups raised in isolation became wild or skittish and therefore much less desirable for experimental purposes. 'Even though the qualities of the Beagle as an experimental dog

are excellent, they can be greatly improved by the researcher having kindness, interest, and practical experience in the handling of this breed'.

Andersen (1955) experimented with partial ventriculo-cordectomy as a means of controlling loud barking in the Beagle, and claimed that if properly performed it did not interfere with the well-being of the dog and gave permanent results (see also Andersen, 1957b). To quote the editors of the U.F.A.W. Handbook on the Care and Management of Laboratory Animals (Lane-Petter, Worden, Hill, Paterson & Vevers, 1967, p.574), however: 'The debarking of dogs by operation on the vocal cords is frowned upon by the Home Office and will probably be made illegal in Britain'.

Andersen & Goldman (1970) have reviewed the growth and development of the Beagle and McFarland (1970) has provided an illustrated account of its topographical anatomy, the special features of which are few. The mesaticcephalic head of the Beagle has a skull about 8.5 cm wide x 14.5 cm long and a skull index of about 59. Singer (1962) illustrated serial sections of the Beagle brain, and data on the breed are included in Lim, Lin & Moffit's (1960) stereotaxic atlas.

'In the Beagles fixed in situ the fissures creating the pulmonary lobes commence near the base of the 5th rib, where the bronchi divide, and radiate therefrom. The fissure between the left diaphragmatic and cardiac lobes extends

from the the base of the 5th rib ventrocaudad to the costochondral cartilage of the 7th rib. On the right side this fissure has about one rib more caudal than on the left... The division into right and left pulmonary arteries occurs at the level of the 4th rib... The heart of the Beagle extends from the 3rd (base of the heart) to the 6th (apex of the heart) rib... Cardiac paracentesis is a common procedure generally performed on the left side between the 4th or 5th intercostal space... at the level of the costochondral junction... The relative heart weight of the Beagle approximates the 0.7 percent mean obtained for 59 dogs of different breeds... The overall dimensions [of the liver] are about 12 cm in length, 11 cm in width, and 12 cm dorsoventrally. The lobular division of the Beagle's liver has the typical canine pattern [Hoerlein, 1965] ... The gallbladder... has a capacity of 10 to 15 ml in the Beagle, and when distended measures about 1.5 cm wide by 5 cm long. Its cystic duct unites with several hepatic ducts to form the common bile duct, which empties into the descending portion of the duodenum about 3 cm distal to the pylorus at a level opposite the 9th or 10th costochondral junction..... The empty stomach, after 24-hour fasting, weighs about 88 g and is situated primarily in the left hypochondriac region, drawn tightly into the gastric depression of the liver... The duodenum arises from the pylorus in the upper right hypochondriac region below the right lateral

hepatic lobe at the level of the 10th costochondral junction... It is about 25 cm long in the Beagle, with its descending portion accounting for slightly more than half this length... The descending portion of the duodenum... terminates at the caudal flexure near the pelvic inlet about 8 cm behind the costal arch at the level of the 6th lumbar vertebra and iliac crests..... The right pancreatic lobe is about 15 cm long, 1 to 3 cm wide and 1 cm thick. The left pancreatic lobe is shorter (10 cm) and wider (4 cm) In both sexes of Beagles ... the dorsal pancreatic duct [is] the larger and [enters] the descending duodenum about 3 cm distal to the entrance of the common bile duct, opposite the right 10th or 11th costochondral junction. The smaller, ventral pancreatic duct penetrates the duodenum separately about 1.5 cm distal to the common bile duct..... The jejunum and ileum constitute the bulk of the intestinal mass... and are about 280 cm long in the embalmed Beagle..... In the Beagle the ileum is about 8 cm long, as defined by the length of [the] antimesenteric vessels... The cecum is located in the right lateral region about 4 to 6 cm caudal to the costal arch under the caudal duodenal flexure and mesoduodenum. In the Beagles examined its apex pointed caudally and laid adjacent to the ileum... The cecum is 4 to 6 cm long and 1.5 to 2 cm in diameter... The ascending colon continues from the ileocolic sphincter cranially about 5 cm... The descending colon is about 12 cm long... The spleen is enlarged 2 to 4 times its normal size in Beagles anaesthetized

with pentobarbital prior to embalming; however, this does not occur when ether is used..... The caudal pole [of the right kidney] extends about 3 to 4 cm beyond the last rib, contacting the right pancreatic lobe and ascending colon... The left kidney is entirely caudal to the last rib, its cranial pole some 2 to 3 cm posterior to the subcostal plane and its caudal pole is 6 to 7 cm beyond that.... The contracted bladder is restricted to the pelvic cavity... and measure about 2.5 to 3 cm in diameter and 3.5 cm to 4 cm in length. The distended bladder passes forward through the pubic region into the umbilical region. In the adult Beagle its capacity approaches 150 to 180 ml..... The left ovary is positioned about 12 to 14 cm caudal to the last rib, usually between the dorsal abdominal wall and the descending colon. The right ovary is located about 8 to 10 cm caudal to the last rib between the dorsal body wall and the duodenum ... The uterine horns are about 10 to 14 cm long, diverging from the body of the uterus about 4.5 cm cranial to the symphysis pubis..... The right adrenal is hook-shaped, about 2 cm long, and is adjacent to the postcava. The left adrenal lies lateral to the aorta and is rounded with a diameter slightly greater than 1 cm. Because of its easier accessibility and longer vessels, the left adrenal gland is generally used in endocrinological studies requiring the collection of adrenal venous blood.... The prostate gland..is ovoid in shape, slightly greater than 2 cm in diameter.... The urethra of the male [has a] total length [of] approximately

25 to 30 cm. Perineal hypospadias was observed in the Beagle along with other urogenital defects (McFarland & Deniz, 1961). The urethra of the female extends about 8 cm from the bladder to the vestibule.... The vagina is about 12 to 14 cm long in the Beagle and 1.5 cm in diameter... The intravaginal cervix extends up to 1 cm into the vagina and is about 0.8 cm in diameter... The vestibule extends about 5 cm from the urethral commissure of the vulva. The clitoris, enclosed within a fossa, is located about 3 cm from the ventral commissure. The vulvar orifice is about 3 cm long, and the dorsal commissure of the vulva is 6 to 8 cm below the anus... The scrotum is located about 8 to 10 cm below the anus. Each testis is about 3 cm long and 2 cm thick.' (McFarland, 1970).

Warner & McFarland (1970) have reviewed the integument of the Beagle and have indicated that it does not show specific breed variation from that of other breeds: it accounts for approximately 16% of the bodyweight.

Papillomata or warts are common in Beagles and become more prevalent with age, affecting only part of the body but most commonly seen on the head and extremities. They may attain a considerable size, and are subject to abrasions or infection. Those on the eyelid may require surgical removal to prevent corneal damage. Squamous cell carcinoma was responsible for some 3% of deaths in Andersen's colony. Some 25% of aged Beagles develop lipomata, which appear as

isolated, globular soft enlargements, varying from 1 to 6 cm in diameter, found most often in the subcutaneous tissue of the torso or proximal extremities, and occasionally within the abdomen. There is no evidence of any malignancy.

Anal gland problems are rare in the Beagle, but external ear infection, usually following parasitic infestation, is quite common and requires appropriate veterinary treatment. Some individuals develop a chronic otitis externa which persists throughout life.

Andersen (1970, pp.226-231) gives values for the Beagle's digestive system. The midportion of the tongue measures about 3.5 cm in width, with an overall length from root to tip of 12 to 13 cm. The oesophagus is about 30 cm in length and 1.5 cm in width. It averages 23g when empty, and has a holding capacity of about 60 ml. A 300 g meal of maize, meat and water, impregnated with barium sulphate, was found to remain in the stomach for 3 to 5 hours. There is no evidence in a Beagle weighing 9 kg that overdistention of the stomach is caused by 400 to 500 ml of fluid. The length of the small intestine varies from 225 to 290 cm and its width is about 1 cm. Ingesta traverse the small intestine in about 1 hour. The holding capacity of this portion of the digestive tract is 200 to 300 ml. The usual capacity of the caecum is 8 to 12 ml. The colon and rectum are about 25 cm in length and have a 200 to 300 ml capacity. Gallbladder capacity is about 10 ml. The pancreas extends along the

duodenum for some 25 to 30 cm, and its width ranges from 3.5 cm in the anterior portion to 1.5 cm posteriorly.

Andersen (1970, pp.524-527) records that both acute and chronic tonsillitis are common in the Beagle, and refers to 7 cases of tonsillar carcinoma observed in animals between 10 and 14 years of age, in which the syndrome was first recognized by difficulty in swallowing and unilateral enlargement of the throat region. Oesophageal abnormalities are rare, but the oesophageal glands commonly have greatly dilated ducts. Constipation, obstipation, diarrhoea and vomiting are seen frequently, as in other breeds, especially in animals maintained out-of-doors. Acute haemorrhagic gastro-enteritis of unknown origin may be fatal, but periodic bloody stools, without much apparent significance, may be encountered in from 1 to 5% of Beagles kept under strict sanitary conditions (Andersen, 1964). Chronic gastritis in the Beagle, as in other breeds, appears to be related to the 'uraemic complex' (Bloom, 1954). Andersen & Hart (1955) recorded an incidence in their colony of 12% in relation to uraemia. Lesions were confined to the mucosa, and included microscopic cysts, fibrosis and round cell infiltration. Primary neoplasms of the stomach were not observed, but two cases of intestinal carcinoma were found, with invasion of the muscularis mucosa of the jejunum.

Andersen found that the most frequent changes in the liver of aged Beagles are congestion, fatty infiltration,

lobular necrosis and metastases. Varying amounts of lobular necrosis have been seen by his colleagues and himself in about one-third of Beagles over 11 years of age. The gross appearance is that of a grayish-white tumour, but histological examination reveals lobules of swollen hepatic cells with pyknotic nuclei, and the lesions usually contain blood-spaces of various sizes, i.e. microscopic telangiectasis. The condition differs from that seen in cattle (Andersen, 1955) in that massive lobular necrosis precedes erosion of the hepatic parenchyma. About one-fourth of all malignant tumours observed in this colony have metastasized to the liver. Almost all Beagles over 12 years of age have shown biliary changes, with thick, tenacious, greenish bile containing dark floccules, and the gallbladder in aged animals invariably exhibits mucosal hypertrophy and cyst formation. The common finding of intrahepatic bile pigment and tenacious bile is thought to be indicative of a sluggish bile flow in senile animals of this breed. Chronic pancreatitis has been found in about 2% of aged animals, sometimes involving the islets of Langerhans, but Andersen refers to only one case of spontaneous diabetes mellitus.

The cardiovascular system of the Beagle does not differ substantially from that of other breeds or mongrel dogs (Detweiler, Buchanan, Fregin & Hill, 1970). The distribution of the ventricular coronary arteries in 37 closely related Beagles was described by Higginbotham (1966), most of the

hearts of which were predominantly left coronary, i.e. the left coronary artery - which originated from the left common coronary or as two independent vessels, the ventral interventricular and left circumflex arteries - reached the dorsal crux of the heart. The right coronary artery originated from the right sinus of valsalva. An artery to the sinoatrial node arose at or near the right marginal branch of the right coronary artery in a few Beagles. Blair (1961) had earlier shown that in most dogs intercoronary anastomoses can be found in vessels about 0.1 mm in diameter (see also Zoll, Wessler, Schlesinger, Freedberg & Blumgart, 1952).

Straus, Wurm, Kositchek & Weiner (1970) found a range in Beagle heart weight of from 46 to 192 g, with an average of 81 g, the ratio being 1.13 g cardiac tissue to 100 g bodyweight. The cardiac output in Beagles compares favourably with that found in other breeds of comparable weight (Detweiler et al., 1970). In dogs weighing from 8 to 12 kg the cardiac output values are 900 to 2,700 ml/minute under anaesthesia and 1,300 to 3,000 ml/minute when standing unanaesthetized. Unpublished data on the mean systolic and diastolic arterial blood pressures and pulse rates in Beagles of various ages, obtained from C.W. Miller, W.J. Tietz & G.F. Smith, of the Colorado State University, Fort Collins, were summarized by Detweiler et al. (1970) and are reproduced in Table XIII. As already indicated, electrocardiographic data and heart rates for Beagles as well as for Corgis at HRC are incorporated in Table XII. (See also Figs. 6 and 7).

Osborne (1972) is currently undertaking at HRC a detailed study of the electrocardiography of the Beagle, the results of which will add considerably to the data for the dog generally compiled by Ettinger & Suter (1970), and to his own preliminary findings (Osborne & Leach, 1971).

Scott & Fuller (1965) recorded the average pulse rates of 70 Beagles at rest as -

150 beats/minute at 17 weeks of age

134 beats/minute at 34 weeks of age, and

137 beats/minute at 51 weeks of age.

The rate in the younger animals was apparently unaffected by the entrance of the experimentalist into the room containing the dogs, but the effect upon the animals of 34 and 51 weeks of age was to increase the rates by 18 and 10 beats/minute respectively. Detweiler et al. (1970) have reproduced data obtained in 1961 by D. R. Young on the effect of treadmill running on pulse rate in Beagles of 1-year-old.

Fox (1963) noted that the heart rate in several breeds, including the Beagle, decreased from birth to 3 days of age, gradually increasing thereafter for some time, due possibly to the gradual development of vagus control over the heart during the postnatal period.

Obese Beagles may succumb to heart failure, and in the colony studied by Andersen (1970, pp. 536-537) about 2% of deaths were attributable to this condition. Such animals had detectable grade III and IV mitral systolic murmurs for

from 2 to 6 years prior to death. There is a high incidence of valvular endocardiosis in this breed, similar to that recorded from other dogs (Detweiler, 1962; Das & Tashjian, 1965). Pericarditis and myocarditis is infrequent, and there is not according to Andersen any particular type of neoplasm that seems to have a predilection for the heart, although metastases are found in this organ and, more frequently, in the tunica adventitia of the aorta, while he has once found a malignant aortic body tumour. Irregularly shaped calcium deposits may be seen in the aortic tunica adventitia in young as well as in old Beagles, and are regarded as being probably without pathological significance.

Straus et al. (1970) have studied the incidence and nature of spontaneous atherosclerotic lesions of the aorta and coronary vessels in 138 Beagles that were affected clinically or died spontaneously between 2,000 and 5,000 days of age, and in a further 17 animals that were sacrificed for normal tissue studies between 250 and 800 days of age. From their series they describe the normal aorta as an extremely elastic organ varying in length from 128 to 238 mm, exclusive of the arch, with an average of 180.5 mm. They found that while the progressive decrease in luminal size for the region of the aortic valve to the bifurcation of the abdominal segment was paralleled by changes in wall thickness there were nevertheless some discrepancies in the lumen:wall relationship. Aortic sclerosis and lipidosi s is first detectable as a slight distortion of the intimal surface

or a relatively firm area of discolouration, and there was a series of gradations to marked lesions, more common in the abdominal than in the thoracic aortic segment.

Straus et al. record a considerably lower incidence of sclerosis in the major coronary arteries than in the aorta, but have encountered some degree of intimal fibrosis and corresponding lipid deposit in about 25% of the animals examined. Another lesion, described as 'mucoid' medial degeneration, and involving primarily the thoracic segment of the aorta was found in 114 of the 155 Beagles they examined.

Ratzlaff (1970) has stated that the Beagle exhibits certain characteristics in relation to the drainage, size, number and location of lymph nodes. His studies were based upon the dissection of 23 adult animals and the main findings are summarized in Table XIV. In some animals he found a hitherto unidentified prefemoral node.

Andersen (1970, p.537) records 6 cases of lymphosarcoma in Beagles that had been X-rayed, and one in a control. He states also that splenic haemangiomata are observed on post-mortem examination of about one-third of all aged Beagles, and that in many animals in this category the spleen shows a diffuse arrangement of the lymphoid follicles and an active haematopoiesis. Adipose tissue is frequently seen, but seems to be of little importance.

Andersen & Schalm (1970) have reviewed the haematology of the Beagle, and a summary of the blood values obtained

from their study of 50 animals of both sexes is provided in Table XV, those from 15 pregnant or lactating females in Table XVI, those correlated with advancing age in Table XVII and those derived from seven different Beagle colonies in Table XVIII. The last table was based partly upon original work, partly upon data supplied by Dr. D.A. Watson, and partly upon the publications of Reber, Kreier, Norton & Malbrotra (1961); Pick & Eubanks (1965); and Michaelson, Scheer & Gilt (1966). The correlation in colony 2 in Table XVIII between PCV and RBC, which in fact was based upon dogs housed indoors, differs considerably from that obtained by Uglialora & Alder (1957), who found that in the 562 blood samples obtained from Beagles kept in outdoor kennels a PCV of 50% had a corresponding erythrocyte count of 8.1×10^6 .

The mean and range of haematological values obtained from more than 2,000 Beagles, all 4 to 5 months of age, at HRC (Noel, 1969; Worden, 1970) are summarized in Table XIX, and age-related changes of values related to red cells in the same colony are summarized in Table XX.

Andersen & Schalm (1970) summarized data from two Beagle colonies to indicate a total blood volume of 82 to 83 ml/kg bodyweight, which is within the range reported for the species (Schalm, 1965), and stated that between one-half and three-fourths of this volume can be exsanguinated.

Three Beagles, 8 to 9 kg in bodyweight, had their carotids severed under barbiturate anaesthesia, and with their body elevated it was found possible to exsanguinate between 4 and 6% of the bodyweight: all three died of respiratory failure, although Zweifach, Chambers, Lee & Hyman (1948) had earlier claimed that dogs can survive this degree of acute blood loss.

Mustard, Secord, Holksema, Downie & Rowsell (1962) reported a clotting defect in an inbred line of Beagles that resembled Factor VII deficiency in man, although without clinical manifestations (see also Garner, Hermoso-Perez & Conning, 1967; Capel-Edwards & Hall, 1968). Dr. Ralph Heywood, of HRC, in his contribution to the book of Appleton & Appleton (1970, p.119) states:

'Increased use of the Beagle in toxicological studies has recently brought to light an inherited blood coagulation disorder in this breed. This defect is due to a deficiency of Factor VII. All the affected animals have appeared clinically healthy, and in no case has spontaneous bleeding occurred, nor has haemorrhage followed blood sampling. Animals with proven Factor VII deficiency, that have been kept for up to two years, have shown no clinical abnormalities. The diagnosis of this condition can only be made by the use of standard laboratory tests. The Quick's one stage prothrombin time is prolonged when Factor VII is deficient, but this can be corrected by the addition of normal canine

serum: the Russell's viper venom time is normal.....
Investigation into the breeding of closed colonies of Beagles would suggest that this defect is a recessive character affecting equally males and females..... The existence of a condition that has no clinical manifestation presents a problem to laboratory animal breeders. It is important that laboratories using Beagles feed back to their breeder any information that would help in eradicating this defect.'

Andersen & Schalm (1970) provide several examples of the haematological response to disease in the Beagle, including that to canine distemper (with a marked lymphopenia), chronic interstitial nephritis (with depressed erythropoiesis and markedly elevated total leucocyte count) and autoimmune haemolytic anaemia.

McKelvie (1970b) has summarized findings on the blood serum chemistry of Beagles, those from his own laboratory at the University of California at Davis having been determined by the procedures outlined by McKelvie, Powers & McKim (1966). His findings from this colony and those from a colony at the University of Rochester (Michaelson, Scheer & Gilt, 1966) are combined in Table XXI. Table XXII summarized McKelvie's findings on changes in serum chemistry values for young Beagles. Data from Beagles at HRC (Noel, 1969; Worden, 1960) are summarized in Table XXIII, and are based upon analyses from over 600 to nearly over 2,600 individual dogs of 4 to 5

months of age. For the purposes of comparison they have been shown together with reported data in man and with other reported data on the dog. Serum alkaline phosphatase levels related to age in Beagles from the same colony are shown in Table XXIV. In Table XXV are shown some comparative data for Welsh Corgis (Pembrokeshire) and for Cocker Spaniels, already referred to, and for Beagles maintained at HRC.

Park, Clarke & Bair (1970) have provided original data for the Beagle on the internal diameters of the bronchi, lung weight and the relative weights of the right and left lung and individual lobes, and these are shown in Tables XXVI, XXVII and XXVIII respectively. They summarize also the clearance rate of various particle sizes from the lower respiratory tract (see Table XXIX), which is a factor to be considered in inhalation toxicity studies. In an earlier publication, Clarke, Park & Bair (1966) had recorded that as much as 50% of a single plutonium aerosol inhalation was present 4 to 5 years later in the tracheobronchial and mediastinal lymph nodes.

Tyree, Glickson & Mickson (1966) recorded values for the pulmonary compliance of 4 Beagles anaesthetized to the point of apnoea. Pulmonary compliance is a descriptive measure of the elastic properties of the lungs and thorax and is in fact the relationship between the pressure change in the airway and the volume of air entering the lungs, i.e. the volume of air change per unit of pressure change.

Their results (mean ml of air) were as follows:

<u>Water pressure (cm)</u>	<u>Compliance value (ml)</u>
5	117 $\pm$ 5.6
10	213 $\pm$ 11.2
15	403 $\pm$ 12.4
20	494 $\pm$ 12.7

Park et al. (1970) preconditioned 20 Beagles to studies of respiration rate and volume, fitting them with masks and studying them at monthly intervals over a 1-year period, restraining them for 5 minutes before each 5-minute testing period. The volume of inhaled air was recorded on a wet test meter and respiration rate with the aid of a thermister on the exhale portion of a two-way valve. They consider respiration rate to be an excellent indicator of respiratory function in the Beagle and that rates above 40 respirations per minute in a well-trained and resting animal to be indicative usually of pulmonary damage. Their results were as follows:

Respirations/min.	23 $\pm$ 2
Tidal volume (ml)	204 $\pm$ 20
Minute volume (l)	4.5 $\pm$ 0.2
Body weight (kg)	13.1 $\pm$ 0.2
Tidal volume/kg (ml)	16 $\pm$ 2
Minute volume/kg (ml)	345 $\pm$ 13

Park, Bair & Clarke (1964) had earlier published data on the oxygen and carbon dioxide contents and pH of the blood obtained from the femoral artery in 20 Beagles at rest, with results as follows:

Oxygen content (ml/100 ml blood)	21.5 $\pm$ 0.7
Oxygen saturation (%)	94.6 $\pm$ 2.0
Carbon dioxide (ml/100 ml blood)	37.7 $\pm$ 1.0
pH	7.416 $\pm$ 0.01

In another study in which 6 Beagles were sampled monthly over a 5-month period, Park et al. (1970) obtained the following results:

pO ₂ (mm Hg)	98.2 $\pm$ 3.7
pCO ₂ (mm Hg)	39.4 $\pm$ 1.8
pH	7.391 $\pm$ 0.006

Andersen (1970, pp. 527-529) records that partial stenosis of the larynx may occur following ventriculocordecotomy or 'debarking', and that in one aged Beagle the lumen of the larynx was found to be about one-third occluded after an operation performed with scissors. He had earlier (Andersen, 1957b) recommended a properly designed biopsy punch for this procedure. Pathological conditions of the respiratory system are not usually directly responsible for fatalities. Varying numbers and sizes of silicotic foci, composed of

macrophages laden with soil particles and a scant amount of connective tissue, are always to be found in the lungs and associated lymph nodes of Beagles that have earth runs. Osseous deposits, never severe enough to be diagnosed clinically, may be found in the lung, and appear to be formed from inhaled particles of bone meal: they show little evidence of an inflammatory reaction. A proliferative pneumonitis may be seen in older Beagles having a lengthy terminal illness. Nodular hyperplasia, reported by Hajdu & Rona (1965) has not been encountered in Andersen's colony. Alternate areas of pulmonary emphysema and atelectasis are not uncommon in senile Beagles, but a generalized emphysema is rare in this breed. The only primary lung neoplasms recorded are those following irradiation (Andersen & Guttman, 1965).

Andersen (1970, pp. 294-295) measured the components of the renal system in Beagles weighing between 9 and 10 kg and found that each kidney weighs about 25 g, with both kidneys together representing about 0.5% of total bodyweight, and that the external measurements of each kidney are approximately 5.3 cm in length x 3.5 cm in width x 2.5 cm in thickness. The ureter measures from 12 to 13 cm in length x approximately 0.2 cm in diameter, while the empty weight of the bladder, which measures some 6 x 8 cm and has a near-full capacity of about 150 ml, weighs from 4.4 to 5.1 g. Guttman (1970) has described the ultrastructure of the glomerulus of the Beagle.

Guttman (1970) studied the kidneys of 110 Beagles that had either been sacrificed or had died, with the wide age range of from 13 to 5,206 days. In the adult animal the renal arteries bifurcate some 2.5 to 3.5 cm from their aortic origin to form the lobar arteries, the pattern and subdivision of which is as described for the dog generally (Arnautovic, 1959). Progressive intercapillary glomerulosis may be detected histologically as early as 6 months old in the Beagle, and intensifies with age. The condition develops independently of extra-glomerular vascular lesions, although such lesions may be associated with severe arteriosclerosis in the late stages. The pathogenesis of progressive intercapillary glomerulosis is not clearly understood, although studies on similar lesions in rodents suggest a possible autoimmune mechanism (Guttman & Bailey, 1965). Farquhar & Palade (1962) consider that the mesangial cell may play an active role in the uptake of plasma constituent.

Renal arteriosclerosis in the Beagle is inconstant and varies in severity among older animals. The accompanying changes resemble those seen in the rat (Spiro, Lattes & Wiener, 1965). There is rarely evidence of subintimal deposits in the small afferent arterioles, as in man, but irregularity of the muscle fibres, with nuclear pleomorphism and an increase in PAS-positive material, is occasionally seen. Atheromatous deposits in the subintimal zones have not been recorded in the Beagle kidney, but with severe

vascular lesions, focal wedge-shaped areas of ischaemic infarction are not infrequent: these areas contain hyalinized glomeruli and atrophic tubules accompanied by dense fibrous tissue and by lymphocytic infiltration of the interstitial tissues at the periphery.

In 2 of the 110 Beagles studied by Guttman, death occurred at 4,373 and 4,567 days of age, respectively, with malignant lymphocytic lymphoma. An interesting observation in his study was the frequent occurrence in older animals of atypical nodular hyperplasia involving the glomerular apparatus, in the form of solid circumscribed nodules varying in size from very small growths to ones that occupied almost all of the glomerular space. In the kidneys of old Beagles, Guttman occasionally found non-encapsulated islands of atypical hyperplasia of the tubular epithelium, associated with advanced arteriosclerosis and occurring in areas of extensive interstitial fibrosis. A few cases of amyloid deposit in the glomerulus and in the medullary stroma were seen, and these could readily be distinguished from the progressive intercapillary glomerulo-sclerosis, there being a decrease in the number of mesangial cells.

Guttman commonly saw cystic changes in the tubules of old Beagles, and recorded 2 cases of advanced subacute diffuse glomerulo-nephritis in animals that were 3,900 and 4,100 days of age respectively, and a single case of focal glomerulo-nephritis at 530 days of age. He considered that interstitial

nephritis, a term that is so frequently employed in describing inflammatory renalopathy in the dog, could not justifiably be applied to the interstitial changes observed in the Beagle: the focal infiltrations of mononuclear cells were associated in his series with glomerular or vascular disease, and were considered as secondary to these, and a diagnosis of a primary type of interstitial nephritis (Bloom, 1939) was regarded as unwarranted. On the other hand he saw a few cases of a primary obstructive ascending pyelonephritis, usually associated with chronic cystitis and obstructive lesions in the lower urinary tract. About 5% of Beagles over 9 years of age had chronic cystitis, the thickened bladder wall exhibiting submucosal congestion and round cell infiltration.

Fox (1957b) records the occasional lack of one kidney in the Beagle. Two such cases were recorded in 1,400 post-mortem examinations, in each case the single kidney being twice the normal size and weight and there being an absence of clinical symptoms.

Guttman (loc.cit.) states that acute cystitis may occasionally be observed in Beagles under 5 years of age. He remarks that primary neoplasms of the bladder are exceedingly rare in this breed.

Hart (1970) states that in the adult Beagle each testis is approximately 3.5 cm long and 2 cm in diameter, and weighs

about 10 g. 'The sensitivity of the testis to alteration of the vascular supply can be illustrated in paraplegic Beagles. Within 2 weeks after spinal transection the testes became markedly atrophic, and microscopic examination of the testicles revealed a marked degeneration of the germinal epithelium. This change was attributed to the effect of spinal shock on the sympathetic vasomotor system, with a consequential alteration of testicular temperature... The prostate gland in the Beagle completely surrounds the urethra and the neck of the urinary bladder and is about 2 cm in transverse diameter.... In our laboratory the paraplegic Beagle maintained on testosterone propionate produced prostatic fluid which could be obtained by evoking ejaculatory reflexes with genital stimulation. Within 30 days after the hormone was withdrawn, the amount of fluid was markedly reduced.... The dog has a copulatory pattern which is rather unique among mammals because of the copulatory lock. Beagles usually remain locked for approximately 15 minutes, but the duration has been observed to vary from 5 minutes to over 60 minutes (observations on about 40 Beagles in over 700 matings).... During erection the transverse diameter of the bulbus glandis changes from approximately 2 cm to a maximum of 5 to 6 cm (averages taken from measurements on 10 adult Beagles). The subsidence of erection is not the converse of tumescence. Detumescence of the bulb takes place sooner than detumescence in the corona and collum...

It is generally believed that contraction of the constrictor vestibuli and constrictor vulvi muscles in the female during mating, presumably occluding venous return from the penis, is responsible for the initiation or maintenance of erection. Observations in our laboratory suggest that this is not the case. For example, complete erection can be evoked in both the intact and paraplegic male dog with appropriate stimulation of penile sensory receptors with no pressure applied to the dorsal veins of the penis [Hart & Kitchell, 1966]. A stimulated copulatory lock can be maintained in paraplegic Beagles for over 40 minutes even though no pressure is applied to the dorsal vein of the penis [Hart, 1967]. Work in our laboratory on the paraplegic male Beagle suggests a mechanism [as follows]. During sexual excitement there is evidently a marked increase in arterial pressure in the branches of the arteries to the penis. The smaller restricted spaces of the c.c. [corpus cavernosum] penis probably engorge almost completely with just this increase in arterial pressure and thus, because of the thick tunica albuginea which surrounds the c.c. penis, this erectile body becomes rather rigid. The cc. penis is firmly anchored to bone at two ends - the ischial tuberosity of the pelvis proximally and the os penis distally. Thus when the c.c. penis becomes rigid from the pelvis to the urethral process this makes it possible for the male to achieve complete intromission during "trial-and-error" thrusting attempts. Complete intromission results in the application of pressure

and rubbing by the female vulva on the sides of the body of the penis just posterior to the bulbus glandis. This stimulation elicits a spinal ejaculatory reflex which is characterized by strong rhythmic contraction of the bulbo-cavernosus muscle and tonic contraction in the ischiourethral muscles... Rhythmic contraction of the transverse fibers of the bulbocavernosus muscle, which surround the urethral bulbs, pumps venous blood from these proximal cavernous spaces of the c.c. urethrae into the distal regions of this erectile body causing rapid filling of the bulbus glandis.... Tonic contraction of the ischiourethral muscle partially occludes venous return from the dorsal vein of the penis and probably aids the rapid filling of the bulbus glandis. The diameter of the bulbus glandis in the fully erect state is so large that it cannot be withdrawn from the vagina. Thus when the male dog dismounts from the female the two remain locked together. During the copulatory lock the rhythmic contractions of the bulbocavernosus muscles become weaker and frequently stop for brief periods. Thus it is probably the tonic contraction of the ischiourethral muscle, by impeding venous return, that is responsible for maintaining the lock. Our preliminary finding of a marked reduction of the lock duration resulting from surgically cutting the ischiourethral muscle in the intact and in the spinal Beagle lends some support to this conjecture.'

Hart adds that work in his laboratory has indicated

that genital receptors on the penis are located more proximally than the glans, and that anaesthetizing the glans with xylocaine or with butacaine sulphate had no detrimental effect upon the mating activity of 6 Beagles. One Beagle, in which the dorsal nerve of the penis was surgically severed about 5 cm proximal to the glans, was able to lock with a female. Studies on 8 castrated male Beagles, each of which had experienced some 30 matings, did not abolish sexual activity for a subsequent period of 16 months, although within a month of castration there were detectable changes, e.g. a drop in the proportion of matings per opportunity and an increased latency from introduction of the female to a complete intromission, with gradual shortening of the duration of the copulatory lock, attributed to a probable effect of androgen withdrawal on the spinal elements mediating the lock. There was no evidence in observations of up to 10 months after castration of any consistent changes in the Beagle in terms of general appearance of the penis or of measurements of the transverse and dorsoventral diameters of the bulbus glandis of the erect penis.

Casarett (1963) reported that the mean volume of the total ejaculate from individual Beagles ranged from 1.4 to 16.8 ml, that the mean pH of Beagle semen ranged from 5.8 to 6.4, and that the mean numbers of sperm per ejaculate ranged from 265 to 841×10^6 .

Andersen (1970, pp. 312-326) has provided an illustrated account of the development and features of the Beagle female reproductive tract, and in general the features appear to bear close resemblance to those recorded for other breeds. Primordial follicles have first been observed at 17 days of age. This is an interesting contrast to the rhesus monkey and to man, in which primordial follicles have been recorded at from the 110th to the 130th and from the 125th to the 155th day of gestation respectively (Van Wageningen & Simpson, 1965). Cessation of prophase meiosis is found to occur in some pups at weaning (38 days of age), but some foci persist in others until 50 or 60 days of age. Oogenesis is regarded as completed by 2 months of age. The primary follicles show evidence of antrum formation at 5 to 6 months of age, comparable with that seen in mongrels of the same age (Raps, 1948). Most follicles undergo atresia but some continue to enlarge so that at 6 months of age the ovary has a definite cortico-medullary function. With the advent of puberty at 10 to 14 months of age some of the vesicles mature.

Oestrus occurs at 7 to 8 month intervals in the Beagle (Andersen & Wooten, 1959), from the age of 9 to 14 months onwards. (Puberty usually occurs earlier in the male Beagle, at from 7 to 8 months according to Andersen, 1970, pp.31-39). Pro-oestrus is indicated by a swelling of the external genitalia, followed in 2-4 days by a sanguineous flow from the vulva which, with the vestibule, becomes enlarged and feels turgid on palpation. The bitch becomes restless and

excitable, drinks and urinates more, and may even exhibit spasms or convulsions that subside with the onset of oestrus. Most female Beagles accept the male within 6 to 10 days from the onset of pro-oestrus. Beach (1965) found that actual acceptance of the male was frequently selective: in his study with 5 male and 5 female Beagles reared together, each of the females would select some males while vigorously rejecting others. Andersen, McKelvie & Phemister (1962) stated that about 10% of the females may require to be held before they will accept the male, even if it is not the first one that has been put with them. The continuous association of both sexes is not conducive to mating (Andersen, 1957). Andersen (1970, p.32) considers that the behaviour exhibited by some females in mounting dogs of either sex and exhibiting pelvic thrusts for a variable length of time is not associated with oestrus, with which he could not detect any correlation, but is generally a reflection of ovarian dysfunction (Walton, 1949; Roberts, 1956). During metoestrus the vulvar discharge becomes scant and dark brown, stopping usually within 7 to 10 days after acceptance. In the animal that has not been mated, anoestrus follows and Andersen (1970, pp.32-33) records that clinical signs of pseudopregnancy have rarely been observed in the Beagle, and that when they occur they are limited to slight mammary development and increased size and reddening of the teats. From data based upon 150 animals whelping between $1\frac{1}{2}$ and 4 years of age he records an average

duration of the oestrous cycle of 220 ± 56 days, the ranges of each stage being: pro-oestrus, 7 to 14; oestrus, 7 to 10; metoestrus, 80 to 90; and anoestrus, 90 to 120 days. Andersen & Wooten (1959) had hitherto recorded a cycle length in 25 unmated Beagles of 228 ± 70 days, with the mean onset of first oestrus at 352 days of age. Andersen (1963) recorded that the cycle lengthened from 1.65 to 1.40 oestral periods a year between $1\frac{1}{2}$ and $7\frac{1}{2}$ years of age.

From the records of nearly 2,000 litters, Andersen (1970, pp.33-34) found that whelping occurred in from 60 to 65 days after acceptance of the male, with an average of 63 days, the same as that recorded by Reinart & Smith (1963). Delayed parturition is believed to be rare in the Beagle, in which fertility is usually considered to be high. Failure to conceive has been recorded as ranging from 3 to 15% in mature animals, although some 50% of older animals may be affected (Andersen, 1965; Reinart & Smith, 1963).

Beagle milk is relatively high in fat (13%) and in protein (9.8%), according to analyses recorded by Luick, Parker & Andersen (1960).

McKelvie & Andersen (1963) found visible lesions in only about 20% of neonatal deaths in their studies on 250 litters. The viral disease described by Carmichael, Squire & Crook (1965) may affect Beagle pups, and strain F-205 was found to be lethal to animals from 1 to 3 weeks of age

(Andersen, 1970, p.37). Fox (1957b) has discussed losses among pups of the breed, which appear to be related to the age of the dam. Andersen, McKelvie & Phemister (1962) found that there were more pups in first than in second litters, but Andersen (1965) recorded a peak in numbers of pups produced at about 3 years of age, with almost a 5% decrease per 100-day breeding period thereafter.

Prolonged anoestrous, pseudo-oestrus and polyoestrus are sometimes observed in Beagle breeding colonies (Andersen & Wooten, 1959) and common non-lethal anomalies include bobtail, prognathism and dwarfism or 'runt' pups. Andersen & Schultz (1958) recorded inherited cataract and associated micro-opthalmia in one out of 1,129 pups. Dr. Ralph Heywood, writing in Appleton & Appleton (1970), states that about 6% of the Beagles examined at HRC have shown some deficiency of pigment in the non-tapetal fundus, with complete absence of pigment in 2%, in which cases the area is red in colour with a network of choroidal vessels. There has not been any evident relationship to coat colours. (The normal part of the tapetum lucidum, or upper part of the ocular fundus of the dog, a triangular, highly reflective and brightly coloured area, is blue, green or yellow in young Beagles, and usually yellow in older animals).

Heywood finds that, in young Beagles examined at HRC, remnants of the hyaloid artery are frequently seen floating within the vitreous attached to the posterior capsule of

the lens, being present in about 20% of animals of 4 to 5 months of age, but in only 4% at 12 months of age. In one strain of Beagles he examined, hereditary cataracts - either unilateral or bilateral, and affecting only part of the lens, but usually the posterior polar region - were present in about 2% at 4 months of age, in rare instances progressing to cause blindness at one year of age. Heywood states that persistence of the pupillary membrane is not an uncommon condition in the Beagle, and is probably hereditary. Heterochromia, or differences in colour within the same iris, is less common in the Beagle than in breeds such as the Dalmation or the Collie, and is not associated with defective vision. Congenital enophthalmos, or sunken eyeball, may be unilateral or bilateral and is associated usually with microphthalmos and with narrowing of the palpebral fissure. It occurs sometimes in the Beagle but entropion and ectropion are rarely encountered in this breed.

Lethal anomalies in the Beagle are rare (Andersen, 1970, pp.37-38), with the exception of hydrocephalus and epilepsy. Epileptic Beagles may not be recognized clinically until several years of age and mild hydrocephalus is sometimes not detected until post-mortem examination.

Diseases of the genital tract are infrequent in the Beagle until old age. Endometritis and pyometra as a cause of death were recorded in 7 only out of 245 Beagles (Andersen, 1970, p.38). The medulla of the ovary in senile Beagles

consists usually of thick-walled blood vessels, between which is dense connective tissue containing a sparse number of epithelioid cells. By contrast to this atrophy, the cortex and rete ovarii undergo hyperplasia, and the surface epithelium of the ovary continues to be active throughout life, as indicated by inward proliferations. Follicles and corpora lutea are often observed on post-mortem examination without macroscopic evidence of pro-oestrus or oestrus in the other parts of the genital system.

Cystadenomas are found in this breed, appearing as a circumscribed mass of cords and tubules, while the most frequent malignant ovarian tumour is the cystadenocarcinoma, involving one or both ovaries and spreading usually by extension into the peritoneal cavity, causing ascites prior to death. Thecal and granulosa-cell carcinomas are less frequent, but can cause widespread metastasis. Andersen (1963a) described the characteristic features of a granulosa-cell carcinoma.

The oviducts and uterus in older Beagles invariably show mucosal hyperplasia, but malignant changes are rare. Anderson (1963b) has described a uterine carcinoma.

Vaginal polyps (leiomyomas or fibromas) have been recorded in some 20% of Beagles over 10 years of age, always located between the vestibule of the vagina and the cervix. They are hard tumours, consisting of compact connective tissue, smooth muscle, or a combination of the two. Transmissible

lymphosarcoma (Feldman, 1932) has not been recorded in Andersen's colony.

The mammary glands of the Beagle are of particular importance to the experimentalist, especially since there are now in being many studies involving the use of the breed for periods of 7 years, with particular reference to the examination of the effects of progestogen-oestrogen combinations that form the basis of the contraceptive pill. (At HRC, one 7-year study in progress involves the examination of a chlorinated hydrocarbon). Sekhri & Faulkin (1970) state that the usual number of functional mammary glands in the Beagle is 9, but that the presence of one or more supernumerary teats is common. These authors have provided an illustrated account of the morphological changes in the glands during pregnancy, lactation, metoestrus and weaning, including electron microscopy of biopsy specimens taken under local anaesthesia from tranquillized Beagles. Some evidence of duct proliferation is to be seen 2 weeks after conception, and by 28 days circular configurations representing terminal ducts and alveoli may be observed. Lobulation is quite distinct by the 42nd day. At this stage of pregnancy a noteworthy feature is the preponderance of lipid droplets, many of which have serrated margins. The mammary gland in the dog two-thirds through pregnancy is in a phase of lipogenesis similar to that of the mouse at a corresponding stage. The fine structure of the myoepithelium in the Beagle resembles that seen in other species, including man (Waugh &

van der Hoeven, 1962), but differs in that the glycogen granules are found among the myofibrils or aggregated in clusters near the nucleus. The ultrastructure of the myo-epithelial cells may be of importance in view of the occurrence of myoepitheliomata in the dog (Mulligan, 1949). By late gestation (58 days) the ultrastructure of the epithelium shows marked changes: the vacuolar component of the Golgi apparatus containing protein droplets has undergone hypertrophy, the endoplasmic reticulum has become more pronounced, with an increased number of attached ribosomes, and there are numerous protein particles and lipid droplets within the alveolar lumen. The mode of milk secretion in the Beagle is merocrine, as is that of the rat (Bargmann & Knoop, 1959) and mouse. Alveolar secretions do not contain large masses of cellular debris during active secretion, but the peri-alveolar or interductal spaces frequently contain a variety of cell types, including mast and plasma cells. The mammary gland of the aged Beagle does not undergo extensive involution, and it is common to find well-developed mammae at post-mortem examination of senile animals. Mammary gland hypertrophy and corpora amylacea are quite common in aged Beagles (Andersen, 1970, pp.532-533). Parenchymal activity of the gland is usually concomitant with ovarian changes.

There is rapid regression of the mammary gland at weaning, virtually complete by the 5th week.

Andersen states that palpable nodules are generally first apparent in female Beagles at 5 or 6 years of age, and that the incidence may reach 50 or 60% by 10 years of age. 'Not all palpable nodules of the mammary glands are tumors, because some detectable tumors regress. Many regressed nodules appear histologically as concretions and indurations... Mammary glands of 280 aging female Beagles were examined clinically semiannually over a 6-year period, during which time approximately 10% of detectable nodules regressed. Palpable nodules which regress are always small in size. In this study about one-half of the palpable mammary tumors were malignant and caused death. The time lapse from recognition of the tumor until death (latency period) varied from 1 to 4 years, with the average time being $1\frac{1}{2}$ years. Histologically these tumors were usually mixed in type, most being composed predominantly of glandular tissue, with varying amounts of connective tissue. The spread of mammary neoplasms in the Beagle is similar to that described for dogs in general [Moulton, 1961]. In brief, these neoplasms spread via lymphatics to the lymph nodes, lung, and adrenals; however, almost any organ can be involved.'

The use of the dog in long-term safety evaluation studies related to drugs and other compounds that act upon the central nervous system renders it desirable that the normal anatomy and physiology of this system be as well

understood as possible, and that there be an adequate appreciation of normal and abnormal behavioural patterns. Fox (1970) has provided an illustrated description of the Beagle brain, paying tribute to Dr. A.C. Palmer, of the School of Veterinary Medicine, Cambridge, whose collaboration with HRC in studies on the effect of hydrazine derivatives is referred to elsewhere in this thesis. In earlier reviews Fox (1964a,b) had indicated that the neuro-ontogeny of the dog is similar to that of other non-precocial mammals such as man and the mouse, and that while its brain at birth is relatively more mature than that of man there is rapid neonatal development of cerebral function, so that at 1 month of age the pup has a near-adult CNS. The dog brain is highly evolved neurophylogenetically, but is less well developed than in more highly specialized mammals. Fox (1966) had also made observations that appeared to correlate neurological change with the period between the 3rd and 4th week of postnatal life, regarded by Scott & Marston (1950) as the time of onset of the critical period of socialization. He has set out the average measurements of the brains of various breeds of dog, and these are reproduced in Table XXX. Stereotaxic data for the Beagle brain have, as already indicated, been published by Lim, Liu & Moffit (1960) and by Singer (1962), and the dog brain generally has been described in detail by Adrianor & Mering (1959) and by Meyer (1964), its gross morphology and development by Fox (1963b), and its dissection by

Miller (1958). Fox (1970), who had also (1963) provided a detailed description of the development of muscle tone and posture in the neonatal dog, has summarized the relevant reflexes, and these are reproduced in Table XXXI.

Fox (1963c) confirmed for the Beagle the development of temperature regulation recorded for the species generally by Jensen & Ederstorm (1955). Pampligione (1963) worked mainly on the Beagle for his study of the development of cerebral function in the dog and recorded that, using subcutaneous electrodes implanted into animals not anaesthetized at the time of test, changes in the electroencephalogram (EEG) during post-natal development occurred not in a continuous but in a step-wise fashion. From birth to 7 days of age, asymmetrical, periodic bursts of activity (5 to 7 and 12 to 15 cycles/second; 30 to 50 μ V), followed by periods of quiescence, were seen in different lobes. Continuous activity was seen after this time and olfactory stimulation caused a weak change in the EEG. By $3\frac{1}{2}$ to 4 weeks of age there was an increase in slow components of the EEG (2 to 5 c/s; frequency 40 to 80 μ V amplitude), and a difference was seen in sleep from that while awake. A K complex was first seen at this time following auditory, visual or tactile stimulation, although no marked differences were recorded between the anterior and posterior regions of the hemispheres. The frontal region had a faster activity but less amplitude. At 7 to 9 weeks of age rhythmic activity became stable and persisted at 4 to 6 c/s until 4 to 5 months of age, at which

time the frequency changed to the adult pattern of 6 to 8 c/s and the amplitude of 20 to 40 μ V.

Fox (1970) discusses several congenital and developmental abnormalities that have been recorded in the brain of the Beagle. Hydrocephaly is more often seen in the brachycephalic breeds but is seen, usually in a mild form in which there is only a slight enlargement of the lateral ventricles. Fox (1964c) described a mild form of hydrocephaly associated with partial agnathia, or shortening of the lower jaw, in a strain of Beagles and described it as a low-grade otocephaly. Epilepsy, possibly associated with the hydrocephaly, was seen in some individuals. A high-grade otocephaly was observed from the same Beagle stock having complete agnathia and partial agenesis of the cerebral cortex.

Idiopathic epilepsy sometimes shows a familial trend in the Beagle. The frequency of seizures usually increases with age or there is a cyclical occurrence (Fox, 1966b), and the syndrome is distinguishable from the nervous symptoms produced by various herbicides, insecticides and rodenticides.

Routine histological examination of the dorsal cerebral hemispheres in aged Beagles reveals varying amounts of neurone degeneration (Andersen, 1970, pp.539-540). Freshly fixed cerebral tissue from about 50% of Beagles of 10 years or more of age shows shrunken and deeply stained neurones similar to those seen in chronic or anoxic cell disease in

man (Cutler & Ederer, 1958). This neuronal degeneration may be fairly uniformly distributed throughout sections of the cerebral cortex, or more extensive areas may appear as a focal degeneration. Neoplastic metastases to the brain are rare, and when they do occur appear in the cerebrum rather than in the cerebellum. Andersen has not observed cerebellar damage of any kind other than the rare metastasis.

The eye is of great importance in toxicology and has probably been the seat of more changes or lesions responsible for the halting of toxicological studies than any other organ, certainly in the case of medicines studied at HRC or elsewhere over the past decade. Andersen (1970, pp. 374-385) has described the anatomical and histological features of the Beagle eye. The compound tubulo-alveolar lachrymal gland in the Beagle is of the serous type. The eyeball in a Beagle of 10 kg bodyweight measures approximately 2.2 x 2.2 cm and weighs some 6g. Dr. Ralph Heywood, writing in Appleton & Appleton (1970, pp.114-118), reference to whose observations on inherited eye conditions has already been made, gives a range of measurements for the Beagle eye as:

Antero-posterior	22-25 cm
Vertical	22-25 cm
Transverse	21-24 cm

- and gives a weight range of 5 to 7 g. Despite general statements to the effect that dogs are myopic, Heywood confirms that the Beagle is emmetropic.

The anterior chamber is deep, occupying one-fifth of the cavity of the eyeball and contains 0.7 to 1 ml of clear fluid.

Shively & Epling (1970) have described the fine structure of the canine eye, with electron microscopy on specimens from Beagles of from 10 to 52 weeks of age.

Andersen (1970, pp. 574-587) describes various pathological conditions encountered in the eye of the Beagle. In the young animal, hypertrophy of the nictatans gland is accompanied by lymphocytic infiltration and greatly dilated ducts. In older animals there is much less evidence of lymphocytic infiltration but even greater dilatation of the ducts, which contain an amorphous substance and necrotic cells in the lumen. Older Beagles may develop tumours of this gland and Andersen refers to two adenomata and one adenocarcinoma in animals over 10 years of age. The case of malignancy appeared similar to hypertrophy of the gland until swelling of the lower eyelid became apparent about a year later. Some 6 months later progressive swelling of the cheek eventually extended into the throat, causing respiratory distress and necessitating euthanasia. The neoplasm involved the retropharyngeal lymph nodes and adjacent tissue.

Corneal opacity and pigmentation are sometimes observed in large Beagle colonies. Complete opacity of the cornea is associated with vascularization of the substantia propria

and leukocytic infiltration. Pigmentation keratitis reveals an abundance of intra- and extra-cellular pigment in the corneal epithelium, while melanin-containing cells may be present in the substantia propria. Heywood (loc. cit.) also refers to melanin deposition in the superficial cornea and sometimes in the deep corneal stroma. Early lesions appear as dark brown or black plaques in the cornea: they are small and discrete with irregular margins, but later may coalesce and obscure vision. The pigment deposits are invariably accompanied by vascularization, but the aetiology of the condition is obscure.

Enlargement of the eyeball and increased intraocular pressure (glaucoma) are seen occasionally in old Beagles, usually but not invariably unilateral. The condition tends to worsen, with damage to the eyeball and a need for enucleation.

Primary tumours of the eyeball are rare in the Beagle. Andersen (1970, pp. 574-580) refers to 3 instances of ocular melanosarcoma in his colony, but says that it could not be determined with certainty whether the condition was a primary tumour or metastasis. In another instance a metastasis of a mammary gland neoplasm was found in the aqueous chambers.

Retinal degeneration is common in Beagles over 8 years of age, and resembles that seen in other breeds of dogs (Parry, 1954). In the Beagle it appears to begin with a

complete loss of rods and cones, followed by disorganization of the remaining layers of the retina. As disorganization of the remaining layers of the retina proceeds, the thickness is reduced to about one-half or less of that seen in the healthy state. When the condition is extensive the retina contains pigment cells. There are differences between this condition and that described by Parry, who recorded the primary lesion as being in the pigment epithelium.

Retinal detachment is infrequent in the Beagle. In the cases observed by Andersen the vitreous body was opaque, the retina showed degeneration of the nuclear layers, and a granular material was located between the separated cones and the pigment epithelium. Such areas occupied about one-fourth of the fundus, with the retina attached peripherally.

The lens surface in the Beagle has suture lines which appear as distinct white lines close to the lens surface (Heywood, 1971). On the lens the pattern is that of an upright 'Y' but on the posterior surface that of an inverted 'Y'. The point at which the suture lines meet is the geometrical centre of the convex surfaces of the lens. By indirect ophthalmoscopy - Heywood employed a Fisons binocular indirect ophthalmoscope in his study of 409 males and 409 females at HRC - 1% of 4-months-old Beagles are seen to show anterior suture lines and 87% to show posterior suture lines. Opacities are frequently seen at the periphery of the lens associated with these sutures: they range from pin-head

size to a coalescence that forms a complete opacity around the equator of the lens. The posterior suture lines and the suture line opacities disappear with age, and at 6 months the proportion with lines has decreased to 17% and at 9 months to 4.5%, while by 1 year old only some 3% show this feature. The suture line opacities have always been found to have disappeared by 8 months of age.

Andersen (1970, pp. 577-580) states that while the posterior lens capsule of the Beagle remains the same thickness throughout life the anterior lens capsule in animals of 8 years of age is some 4 to 5 times thicker than in the young adult. This thickening begins immediately to the equator of the lens, with an indipping of the underlying epithelium and lens fibres. Andersen states also that varying sizes and shapes of lenticular vesicles are quite common in Beagles, and are usually located in the outer portion of the lens at the equator or posteriorly. They contain a lightly staining eosinophilic amorphous substance, surrounded by normal fibres, are presumably developmental in origin and are of little consequence. They probably do not impair vision because they are small in size and usually of peripheral location. Of 118 Beagles more than 8 years old examined by Andersen, partial lenticular abnormalities were detected in 30 animals, i.e. 22%. The opacities were subcapsular, and involved usually the posterior portion of the lens. Aberration of the lenticular fibres and nuclei

were the usual findings, and the capsule itself did not appear to be defective. Lesions were rarely observed in the inner portion of the lens.

Heywood & Partington (1971) recorded superficial corneal vascularization in one Beagle at HRC maintained on a riboflavin-deficient diet and corneal opacities in 3 out of 7 animals receiving a diet that was sub-optimal in riboflavin.

Michaelson (1970) has reviewed the endocrine system of the Beagle, and the range of weights of the thyroid, adrenal and pituitary or hypophysis in this breed reported by various authors (Mayer, 1947; Jackson & Cappiello, 1964; Hodge, Panner, Downs & Maynard, 1965; Marton, Greselin, Givner & Voith, 1964; Hadidian & Pawlowski, 1964; Pick & Eubanks, 1965) have been extracted and summarized by him and tabulated by comparison with studies on mixed breeds, of which some 21% were actually Beagles (White & Foust, 1944; Francis & Mulligan, 1949; Hewitt, 1950; Randolph & Getty, 1951; Haensly, Jermier & Getty, 1964; Haensly & Getty, 1965): this is reproduced as Table XXXII.

Michaelson (1970) states that in 37 male and female Beagles ranging from 2 to 14 years of age the plasma protein bound iodine (PBI) level was 2.3 ± 0.8 $\mu\text{g}/100$ ml, without any evidence of correlation with age. In 17 Beagles, 2 to

9 years of age, the PBI was 2.5 ± 0.05 $\mu\text{g}/100$ ml, while in 13 adult Beagles the PBI was 2.1 ± 0.04 $\mu\text{g}/100$ ml. The iodine intake of these animals was 430 μg . Michaelson quotes values for free thyroxine of 2.92 ± 1.10 $\text{m}\mu\text{g}$ for adult Beagles, and of 3.80 ± 0.80 $\text{m}\mu\text{g}$ for animals of 6 months of age, compared with 3.53 ± 0.34 $\text{m}\mu\text{g}/100$ ml in mongrels.

Rabinowitz, Banks & Greenberg (1964) employed a Sephadex chromatographic method to determine tri-iodo-thyronine uptake by the erythrocytes as a measure of thyroid function in the Beagle, and recorded uptakes ranging from 64 to 79%, with a mean of 71%, in the normal animal.

Greelish & Reineke (1961), in a study involving 12 animals, reported an uptake of I^{131} of 18 to 19% within 24 hours by the Beagle thyroid. Uptake was maximal by the 3rd to 4th day, after which there was a decline of 6 to 7% per day. Riddell & Robinson (1965) recorded a thyroid uptake at 24 hours of $15.6 \pm 1.1\%$ in 30 female Beagles over 10 months of age, and one of $17.0 \pm 1.8\%$ in 9 males of corresponding age. In 52 Beagle-Cairn Terrier crosses the value was $12.5 \pm 0.6\%$. The maximum concentration of I^{131} occurred between 48 and 72 hours after its injection, and in 5 males that were deficient in Factor VII the values at this time were $29.3 \pm 1.9\%$.

Goyings, Reineke & Schirmer (1962) employed I^{131} uptake, together with physical examination, to evaluate thyroid function in several breeds of dog, including the Beagle.

The 'thyroid-to-thigh' ratio of the counts was 28:1 in 22 control animals and 3.56:1 in 40 cases of hypothyroidism.

Berson, Yalow, Sorrentino & Roswit (1952) measured the plasma clearance and thyroid uptake of I^{131} in Beagles and recorded thyroid clearance values of 0.73 ± 0.32 and of 0.80 ± 0.36 litres, respectively, at 1 and 2 hours after injection.

Michaelson (1970) refers also to studies on the effect of thyrotropin administration on PBI, on I^{131} uptake and on circulating cholesterol levels in Beagles of 2 to 14 years of age, with a correlation coefficient of 0.986 between injected thyrotropin and PBI. He quotes serum cholesterol levels of 218 ± 55 mg/100 ml in euthyroid Beagles of 10 months of age and levels of 172 ± 34 mg/100 ml in those of from 2 to 14 years of age: Bloom (1960) cites a range of from 140 to 210 mg/100 ml for the normal dog, without reference to breed.

Collins, Garrett & Johnston (1962) found that the blood calcium level in young Beagles is increased by steroid therapy, which appears to inhibit the incorporation of calcium into bone and to accelerate the absorption of calcium from bone. Tonelli, Haynes, Heyder & Ringler (1964) employed Beagles of 10 to 15 months of age for an assay procedure based on the eosinopenic response to corticosteroids administered by mouth or intravenously.

Michaelson (1970) refers to a case of Cushing's syndrome in a female Beagle. Severe dermatosis first appeared at $6\frac{1}{2}$ years of age and continued sporadically throughout the remaining 2 years of life, requiring occasional treatment. Alopecia, thickened skin and bouts of ataxia became progressively worse. Severe pain over the kidney was evident. At $8\frac{1}{2}$ years of age anorexia was complete and the animal was sacrificed. Findings included oedema, varying amounts of haemorrhage, parenchymal hypertrophy in the lymph nodes, liver, pancreas, kidneys and cerebrum, and cystic follicles and adenoma in the ovary. Serial sections of the anterior pituitary stained with Koneff's method revealed atrophy of chromophobe cell types. Acidophils were normal in appearance and numbers. Basophils were normal in appearance and numbers. Chromophobe cells, when identifiable, showed hydropic degeneration, and in many cases the presence of chromophobes was indicated by an enlarged, clear space. Adrenal enlargement was due to cortical hypertrophy, with large, pale-staining cells.

McGrath (1960) described a case of craniopharyngioma in a 3-years-old male Beagle. The animal had diabetes insipidus, persistent alopecia, gradual increase in body size, progressive listlessness and increased appetite, while its testes were small. The craniopharyngioma occupied the anterior portion of the infundibular stalk. Loss or degeneration of cells of the hypothalamic nuclei was evident. Pick

& Eubanks (1965) reported pituitary cysts in 3 of the 37 Beagles they subjected to post-mortem examination. Saunders, Stephenson & McEntee (1951) described an infundibuloma in a 12-years-old crossbred male Beagle with diabetes insipidus and adiposogenital syndrome. Michaelson (1970) refers to an adenocarcinoma of the pars distalis of the pituitary in a 12-years-old female Beagle that had received 100 R, 250 kVp X-rays at 8 months of age, as well as to X-ray induced hypothyroidism in animals of the same breed of 1 to 5 years of age.

Tucker (1962) reported a 16.2% incidence of chronic thyroiditis in 167 laboratory Beagles approximately 2 years of age. There were no gross lesions or clinical signs of thyroid dysfunction, but the histological evidence included nodular and diffuse infiltration by lymphocytes, plasma cells, macrophages and neutrophils, the degree of involvement varying from scattered foci to diffuse infiltration of the entire section. Michaelson (1970) states that chronic thyroiditis has been recorded in 10% of young adult Beagles maintained at the laboratories of the U.S. Food and Drug Administration and in 20% of those over 10 years of age in the colony of the University of California at Davis. Pick & Eubanks (1965) reported thyroiditis in 3 of the 37 Beagles already referred to.

Andersen & Johnson (1964) described a carcinoma of the thyroid in an 11-years-old female Beagle, this being the only

thyroid tumour recognized in a pathological study of over 250 aged Beagles. A circumscribed, hard enlargement was palpated in the left thyroid region at 9 years of age, and at 10½ years had grown to approximately 5 cm in diameter. A globular encapsulated tumour, weighing 13.5 g, of the left thyroid was revealed on dissection, and appeared histologically to be a well-differentiated adenocarcinoma. There were several less well-differentiated metastases. The right thyroid was atrophic.

Pearson, Dellman, Barrier, Case & Collier (1965) reported primary hyperparathyroidism in a 3-years-old female Beagle. The serum alkaline phosphatase and calcium levels were raised, and the histologic diagnosis was that of an active clear chief cell hyperplasia.

Andersen (1970, p.536) states that the adrenal glands may show extensive necrosis in Beagles dying from shock, and that hyperplasia of the cortex is frequently observed in those suffering from an extended terminal illness, when there may be bulging through the capsule of the gland. Ectopic haematopoietic foci are sometimes encountered.

The widespread use of the Beagle renders background information on survival and on age-changes of considerable importance to the experimentalist. At HRC the vast majority of long-term studies are ended by the time that the animals are some two-and-a-half years of age but, as already stated,

there is one 7-year study in progress and elsewhere many experiments involving progestational steroids or hormonal combinations are in progress, while investigations of similar length are accepted by the relevant authorities in some countries, including the United States of America and Great Britain, as a suitable part of the evidence necessary to investigate potential carcinogenicity. The survivability of Beagles under so-called natural and under laboratory conditions has been investigated by Andersen & Rosenblatt (1965). The median survival under 'natural' or field conditions was 7.5 years, whereas in the laboratory it was much greater. Table XXXIII shows the probability of survival of control female Beagles entered into a life-span study at one year of age, and indicates a mean life-span of 12.5 years in the protected environment of the laboratory. Of the 45 animals that died during a life time experiment initially involving 60 Beagles (Andersen, 1970, pp. 541-544), the precipitating cause in 24 cases was neoplasia, originating mainly from the reproductive system. By contrast, renal damage is given as the primary cause in other breeds (Bloom, 1954) while according to the compilations of Mulligan (1949), Cotchin (1959) and Moulton (1961) neoplasms of the skin are more numerous than those of the genital system.

The subjective signs of ageing in the Beagle include lethargy, wrinkling of the skin, greying of the hair, dental defects and changes in bodily conformation. Wrinkling of the skin becomes apparent in areas such as the labial

commissure, throat, axilla and flank where it is most mobile, and often appears thickened and pigmented. Greying of the hair begins usually at about 6 years of age and affecting at first the lips, eyelids and distal extremities. There is, however, considerable individual variation. Tooth wear down to about half the dental table is present in many animals over 10 years of age, and in some instances the wear extends down to the gingival margin. Tartar accumulation, periodontal disease, tooth abscess, sinusitis and tooth loss are more common in animals that show little tooth wear. Rosenberg, Rehfeld & Emmering (1966) studied periodontal disease in a Beagle colony and concluded that diet and genetic predisposition were the main factors involved.

The angle of the pastern of the Beagle decreases with age, the head is carried lower, and the lumbar region broadens.

In an effort to help to distinguish physiological from pathological ageing, Nagode, Frajola & Loeb (1966) obtained baseline data for 5 tissue enzymes in mature Beagles, while reference has already been made to the changes of thyroid and adrenal gland weights with age (Haensly et. al., 1964; Haensly & Getty, 1965). Although not specific for the Beagle another age indicator is the development of lipofuscin, present from about 4 years of age in the nervous system and increasing in amount throughout life (Whiteford & Getty, 1966; Getty, 1966).

Solarz (1970) has reviewed the behaviour of the Beagle, which James (1953) classified along with Basset Hounds and St. Bernards as an 'inactive' type, by contrast with the 'active' Whippet and Saluki and with Terriers. When 22 Beagles were compared with approximately equal numbers of other breeds, including Basenjis, Cocker Spaniels, Wire-haired Fox Terriers, Shelties and hybrids, they were found to vocalize more but to compare favourably with tests such as reaction to the leash, baulking and interference with the trainer's movements. Scott & Fuller (1965), however, state that the Beagle is restless under restraint.

Elliott & Scott (1965) compared the ability of 29 Beagles to traverse a complex maze with that of various other breeds. The Beagles at the outset engaged in random exploration which led them into many blind alleys, but despite this poor start their persistence prevented them from forming stereotyped incorrect behaviour and their comparatively high degree of motivation produced a rapid improvement so that they ended with the best performance.

The 'whirling behaviour' referred to in 3:3 as occurring in Scottish Terriers that had been isolated for long periods has been noted by Solarz (1970) in Beagles under both restricted and non-restricted conditions, and tended to occur when excitement was high. In some dogs it consistently occurred in a mild form prior to feeding, particularly if the animals were forced to wait while others were being fed.

Beagles were employed by Melzack (1962), in a study that indicated that early perceptual deprivation reduced the ability to learn simple discriminations of brightness differences. In a study by Melzack & Burns (1964), dogs that had been subjected to environmental restriction for a period of 10 months or longer showed deficiencies in pain sensitivity and in avoidance-learning, and failure to respond emotionally to fear-producing auditory stimuli. The dominating feature of behaviour was an increase in excited activity, and in a few cases 'whirling fits'. A male when released showed increased excitement activity in response to a female in oestrus, but its behaviour was undirected. The present author has witnessed similar excitable and undirected behaviour in male dogs of other breeds, including the Scottish Terrier, that have been maintained as pet animals within a household and have suddenly found access to females, or indeed to other males, while Scott (1964), not writing specifically of Beagles, has found that a socially restricted female, although in oestrus, exhibits immature, playful behaviour towards males. In an investigation of the social responses of Beagle puppies toward a handler and towards a companion test puppy, chlorpromazine was found to depress the activity level and to facilitate the extinction of learned avoidance behaviour (Fuller, Clark & Waller, 1960; Waller & Fuller, 1961).

Solarz (1970, p.463) has found that when two adult female Beagles are penned together, fighting tends to diminish if a clear dominance-submission relationship is recognized by both animals; but that severe fights may occur if the members of the pair are alike in size, weight and past experience. The same author (1964, 1965) has made detailed studies of these dominance-submission relationships and has shown that when oxtail bones are put into the pen the strongly dominant animal will control them all, even if they are spread about the area: the submissive animal cowered in a corner and was at times attacked without provocation. In other pairs, however, a 'parallel possession' relationship was worked out without much evidence of friction. Weight was the main factor correlated with dominance, and in many instances the dominant animal worked out tactics that enabled it to consume some of the submissive dog's food. Bodyweight differences tended to increase with time. These considerations would seem to be of considerable significance in relation to paired Beagles on long-term studies.

Pawlowski & Scott (1956) found that Beagle puppies developed fewer complete dominance interactions than did either Fox Terriers or Basenjis, and that there was no dramatic male-over-female dominance among Beagles. James (1951) found that when Fox Terriers were raised together with Beagles, the Terriers consistently won the tests for dominance over the food pan, and sired all of the pups of

both breeds. When each animal was given freedom to choose one of a pair of available dogs for its companion, Beagles chose Beagles rather than the dominating Fox Terriers, while Fox Terriers tended to choose Beagles.

DiMascio (1959) observed some interesting within-breed behavioural differences in the Beagle in relation to body type, in tackling delayed-response tasks. The stockier or less ectomorphic animals could delay a response for a longer period of time, required fewer cues for proper orientation, and were less active during the imposed delay period. From a study of several breeds, it would appear that performance in tests of this type is facilitated by an ability to inhibit overt motor responses, and that the characteristics are present as early as 10 to 16 weeks of age (Fuller & Gillum, 1950).

'Beagles are particularly friendly and their affectional responses are readily released toward a familiar human being. They are equally sensitive to strangers as shown by excessive barking when a stranger enters the kennel area. Plans to observe behavior should take both of these characteristics into account. Maintaining a distance, using blinds, and keeping movements routine are key factors for good observation' (Solarz, 1970, p.465). Solarz concludes also that the Beagle's behavioural attributes combine well with its physical features to make it one of the preferred breeds of research, and that behavioural variables, even when not the

primary concern, can be extremely critical in the planning, conduct and interpretation of any animal research.

Anderson & Brady (1971) employed Beagles as well as mongrels in monitoring studies carried out continuously for an hour prior to performance on a free-operant shock-avoidance task, and recorded a sustained and significant increase in blood pressure and a sustained and significant decrease in heart rate.

The Beagle's intestinal flora appears to resemble that reported for other breeds, but Clapper (1970) has not isolated Salmonella species and has found Proteus species only infrequently. Streptococcus mitis and Escherichia coli form a stable part of the flora, with other types of streptococci, staphylococci, Gram-positive aerobes and anaerobes and Bacteriodes usually present but varying in numbers from month to month. Clapper has isolated organisms with the characteristics of mycoplasma, from the throat and rectal swabs of apparently healthy Beagles, and has evidence of the existence of viruses in the faeces and in nasal or throat swabs, but indicates that much more work on these aspects is required.

Schultz (1970) has reviewed the genetics of the Beagle and Moore (1970) its cytogenetics. Special techniques described in relation to the Beagle include whole-body counting (Richmond & Furchner, 1970).

Nutritional studies involving the Beagle are referred to in 4:3, food consumption and bodyweights at HRC being depicted in Tables XXXIV and XXXV.

TABLE XIII

MEAN SYSTOLIC, DIASTOLIC, PULSE AND BLOOD
PRESSURES (mm Hg) AND PULSE RATES OF BEAGLES
OF VARIOUS AGES

No. Beagles	Age (months)	Aortic valve			Thoracic aorta			Abdominal aorta			Ileo-femoral			Mean pulse rate
		S	D	BP	PP	S	D	BP	PP	S	D	BP	PP	
5	2-8	91 ±7	57 ±13	75 ±9	37 ±14	87 ±4	53 ±12	72 ±10	34 ±12	103 ±24	48 ±18	69 ±16	54 ±8	178 ±12
7	7-10	99 ±27	67 ±23	81 ±25	32 ±12	109 ±32	67 ±25	84 ±28	40 ±11	125 ±34	70 ±28	87 ±31	55 ±10	124 ±20
7	23-59	114 ±21	84 ±21	93 ±20	30 ±13	112 ±15	72 ±9	84 ±12	40 ±9	120 ±26	69 ±6	84 ±9	51 ±22	127 ±22

S = systolic pressure
D = diastolic pressure

BP = blood pressure
PP = pulse pressure

TABLE XIV

The Number, Size, and Occurrence of the Lymph Nodes of the Beagle

Lymph node	Percent occurrence	Mean size (cm)	Size variation (cm)	Average number per side	Variations in number per side
Parotid	100	1.0 x 0.7	0.7 x 0.2, 1.6 x 0.9	1	1-2
Mandibular	100			3	2-6
Dorsal	100	1.2 x 0.8	0.3 x 0.2, 1.6 x 0.9	1	1-2
Middle	36	1.2 x 0.7	0.5 x 0.3, 2.0 x 0.8	1	0-2
Ventral	100	1.3 x 0.8	0.7 x 0.4, 1.9 x 1.0	1	1-2
Medial retropharyngeal	100	3.2 x 1.3	2.3 x 1.1, 4.2 x 1.4	1	none
Lateral retropharyngeal	31	0.5 x 0.3	0.3 x 0.1, 0.6 x 0.4	1	0-2
Superficial cervical	100			2	1-3
Dorsal	100	1.6 x 0.9	0.5 x 0.2, 2.1 x 1.2	1	1
Ventral	86	1.5 x 0.8	0.4 x 0.2, 2.0 x 1.5	1	0-2
Deep cervical	77			2	0-4
Cranial	62	0.4 x 0.2	0.2 x 0.1, 0.6 x 0.3	1	0-1
Middle	33	0.4 x 0.2	0.2 x 0.1, 0.7 x 0.4	1	0-1
Caudal	33	0.2 x 0.1	0.1 x 0.1, 0.5 x 0.3	1	0-2
Axillary	100	1.4 x 0.9	0.9 x 0.7, 2.0 x 1.8	1	1-2
Accessory axillary	53	0.9 x 0.6	0.3 x 0.2, 1.5 x 1.0	1	0-2
Sternal	100	0.7 x 0.4	0.3 x 0.3, 1.4 x 0.7	1	1-3
Intercostal	100			2	1-4
Cranial	75	0.3 x 0.2	0.1 x 0.1, 0.5 x 0.3	1	0-2
Caudal	75	0.3 x 0.2	0.2 x 0.1, 0.7 x 0.6	1	0-2
Mediastinal	100			2	1-5
Cranial	100	0.7 x 0.4	0.1 x 0.1, 1.1 x 0.6	1	1-2
Middle or caudal	67	0.7 x 0.4	0.2 x 0.1, 1.5 x 0.7	1	0-3
Tracheobronchial	100			...	...
Left	100	1.4 x 0.6	0.9 x 0.4, 1.8 x 0.8	1	none
Middle	100	2.5 x 0.8	1.8 x 0.7, 2.8 x 0.9	1	none
Right	100	1.1 x 0.6	0.7 x 0.4, 1.8 x 0.8	1	1-2
Bronchopulmonary	75			1	0-3
Left	25	0.4 x 0.3	0.2 x 0.2, 0.5 x 0.3	1	0-2
Right	75	1.2 x 0.5	0.8 x 0.4, 1.7 x 0.6	1	0-1
Lumbar	100			3	1-7
Cranial	100	0.7 x 0.3	0.3 x 0.1, 1.1 x 0.4	1	1-4
Middle	69	0.3 x 0.2	0.1 x 0.1, 1.1 x 0.3	1	0-3
Caudal	62	0.5 x 0.4	0.2 x 0.1, 1.0 x 0.6	1	0-1
External iliac	100	1.9 x 0.7	1.0 x 0.4, 2.8 x 0.9	1	1-2
Internal iliac	90	1.3 x 0.6	0.7 x 0.5, 2.6 x 0.8	1	0-1
Sacral	87			...	...
Left	73	0.4 x 0.3	0.1 x 0.1, 1.0 x 0.4	1	0-2
Middle	60	0.4 x 0.3	0.2 x 0.1, 0.7 x 0.4	1	0-2
Right	66	0.5 x 0.3	0.1 x 0.1, 1.2 x 0.4	1	0-2
Deep inguinal	65	0.4 x 0.3	0.1 x 0.1, 0.8 x 0.5	1	0-2
Hepatic	100	1.4 x 0.6	0.8 x 0.5, 2.6 x 0.7	1	1-3
Splenic	100			2	1-4
Proximal	100	0.5 x 0.3	0.1 x 0.1, 1.0 x 0.5	1	1-3
Distal	87	1.2 x 0.5	0.4 x 0.3, 3.0 x 0.6	1	0-1
Mesenteric	100			2	1-4
Left					
Cranial	100	6.5 x 1.3	1.7 x 0.7, 8.1 x 1.6	1	1-2
Caudal	56	1.0 x 0.7	0.3 x 0.2, 5.5 x 1.5	1	0-2
Right					
Cranial	100	4.2 x 1.3	1.5 x 1.2, 6.0 x 2.1	1	1-2
Caudal	69	2.8 x 1.1	0.7 x 0.4, 5.3 x 2.1	1	0-2

TABLE XIV (continued)

Lymph node	Percent occurrence	Mean size (cm)	Size variation (cm)	Average number per side	Variations in number per side
Colic	100			...	...
Left	100	0.8 x 0.5	0.3 x 0.2, 1.5 x 0.8	3	2-4
Middle	100	0.8 x 0.5	0.2 x 0.1, 2.2 x 1.3	2	1-4
Right	100	1.1 x 0.7	0.2 x 0.1, 2.3 x 1.8	3	1-5
Gastric	100	0.8 x 0.5	0.2 x 0.1, 1.6 x 0.7	1	1-3
Duodenal	100	0.9 x 0.5	0.4 x 0.3, 2.1 x 0.6	1	1-3
Omental	64	0.5 x 0.3	0.3 x 0.2, 0.7 x 0.4	1	0-1
Popliteal	100	1.3 x 0.9	0.5 x 0.5, 2.2 x 1.2	1	1-2
Medial femoral	33	0.3 x 0.2	0.1 x 0.1, 0.6 x 0.3	1	0-3
Superficial inguinal	100			2	1-3
Cranial	100	1.5 x 0.7	0.2 x 0.1, 3.0 x 1.1	1	none
Caudal	67	1.0 x 0.6	0.3 x 0.2, 2.0 x 1.2	1	0-2
Prefemoral	22	0.3 x 0.2	0.1 x 0.1, 0.7 x 0.5	1	0-3

TABLE XV

Blood Values From Male and Female Beagles Through Puberty to Early Adulthood

	Age in Days											
	300		360		420		480		540		600	
	M	F	M	F	M	F	M	F	M	F	M	F
Erythrocytes (10^6)	7.07 $\pm$ 0.5	6.98 $\pm$ 0.7	6.98 $\pm$ 0.6	6.69 $\pm$ 0.5	7.0 $\pm$ 0.5	6.83 $\pm$ 0.5	6.95 $\pm$ 0.5	6.67 $\pm$ 0.5	6.94 $\pm$ 0.5	6.70 $\pm$ 0.5	6.95 $\pm$ 0.4	6.67 $\pm$ 0.5
Packed cell volume (%)	53.8 $\pm$ 2.6	54.2 $\pm$ 3.6	53.9 $\pm$ 2.7	53.2 $\pm$ 3.1	54.2 $\pm$ 2.5	53.9 $\pm$ 3.2	54.6 $\pm$ 3.1	53.9 $\pm$ 3.6	54.6 $\pm$ 2.9	53.2 $\pm$ 3.7	55.0 $\pm$ 2.6	53.6 $\pm$ 4.2
Hemoglobin (g/100 ml) [†]	16.8 $\pm$ 0.9	17.0 $\pm$ 1.1	17.1 $\pm$ 0.9	16.8 $\pm$ 1.0	17.1 $\pm$ 0.8	17.0 $\pm$ 1.0	17.2 $\pm$ 1.0	16.8 $\pm$ 1.1	17.2 $\pm$ 1.0	16.8 $\pm$ 1.1	17.4 $\pm$ 0.9	16.9 $\pm$ 1.3
Leukocytes (10^3)	12.9 $\pm$ 2.9	13.1 $\pm$ 3.0	12.9 $\pm$ 2.4	12.4 $\pm$ 2.4	13.0 $\pm$ 3.6	13.2 $\pm$ 4.8	12.5 $\pm$ 2.5	11.6 $\pm$ 2.5	12.9 $\pm$ 2.1	12.1 $\pm$ 3.2	14.6 $\pm$ 4.0	13.8 $\pm$ 3.7
Neutrophils	7.9 $\pm$ 2.1	7.5 $\pm$ 0.3	7.9 $\pm$ 1.9	7.6 $\pm$ 2.0	8.8 $\pm$ 2.7	8.0 $\pm$ 2.6	8.6 $\pm$ 2.0	7.6 $\pm$ 2.2	8.6 $\pm$ 1.8	7.9 $\pm$ 2.3	10.3 $\pm$ 3.4	9.8 $\pm$ 3.4
Bands	0.5 $\pm$ 0.3	0.6 $\pm$ 0.3	0.5 $\pm$ 0.3	0.5 $\pm$ 0.3	0.5 $\pm$ 0.3	0.5 $\pm$ 0.3	0.5 $\pm$ 0.2	0.4 $\pm$ 0.2	0.5 $\pm$ 0.2	0.4 $\pm$ 0.2	0.4 $\pm$ 0.3	0.4 $\pm$ 0.3
Eosinophils	0.5 $\pm$ 0.3	0.3 $\pm$ 0.4	0.6 $\pm$ 0.4	0.6 $\pm$ 0.4	0.5 $\pm$ 0.4	0.6 $\pm$ 0.7	0.5 $\pm$ 0.3	0.5 $\pm$ 0.3	0.5 $\pm$ 0.3	0.6 $\pm$ 0.4	0.4 $\pm$ 0.3	0.5 $\pm$ 0.5
Lymphocytes	3.1 $\pm$ 1.2	2.9 $\pm$ 0.8	2.8 $\pm$ 0.8	2.9 $\pm$ 0.9	2.8 $\pm$ 0.8	2.8 $\pm$ 0.8	2.6 $\pm$ 0.8	2.7 $\pm$ 0.8	2.9 $\pm$ 0.9	2.9 $\pm$ 1.0	3.1 $\pm$ 1.0	2.9 $\pm$ 1.1
Monocytes	0.2 $\pm$ 0.0	0.2 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.4 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	0.3 $\pm$ 0.0	3.0 $\pm$ 0.0	0.3 $\pm$ 0.0

TABLE XVI

BEAGLES: BLOOD VALUES DURING

GESTATION AND LACTATION

(MEAN OF 15 ANIMALS)

Weeks	Gestation				Term	Lactation		
	2	4	6	8	0	2	4	6
Erythrocytes (10^6)	8.85	7.48	6.73	6.23	4.53	5.13	5.65	6.15
Packed cell volume (%)	53	47	44	37	32	34	38	42
Hgb (g/100 ml) (Cmh)	19.6	16.4	14.7	13.8	11.0	11.7	12.8	13.4
Sed. rate (corrected)	0.6	11.0	13.0	14.0	12.0	14.0	14.0	13.0
Leukocytes (10^3)	12.0	12.2	15.7	19.0	18.9	16.9	17.1	15.9

TABLE XVII

BEAGLES: BLOOD VALUES WITH
ADVANCING AGE *

	Age in months					
	12-24	25-36	37-48	49-60	61-72	73-84
Packed cell volume (%)						
No. of samples	158	143	145	125	84	34
Mean $\pm$ S.D.	49.9 $\pm$ 4.1	50.8 $\pm$ 4.6	50.0 $\pm$ 4.6	51.2 $\pm$ 4.7	50.0 $\pm$ 5.1	53.5 $\pm$ 6.2
Hemoglobin (Cmh)						
No. of samples	69	87	95	116	85	36
Mean $\pm$ S.D.	14.8 $\pm$ 2.4	16.0 $\pm$ 4.3	17.5 $\pm$ 1.9	17.4 $\pm$ 1.8	17.1 $\pm$ 2.0	17.8 $\pm$ 2.5
Sed. rate (corrected)						
No. of samples	123	126	141	126	86	35
Mean $\pm$ S.D.	1.0 $\pm$ 3.2	0.7 $\pm$ 2.0	0.4 $\pm$ 2.0	0.4 $\pm$ 2.0	0.4 $\pm$ 2.0	0
Leukocytes (10^9)						
No. of samples	168	139	124	111	83	22
Mean $\pm$ S.D.	11.6 $\pm$ 3.4	11.1 $\pm$ 3.3	11.3 $\pm$ 2.9	10.4 $\pm$ 2.1	11.0 $\pm$ 2.0	10.0 $\pm$ 1.8

* Sample number varies because of differences in ages when bled on a quarterly basis. A complete blood count was not done on each sample. Beagles kept in outdoor pens.

TABLE XVIII

BEAGLES: BLOOD VALUES BASED UPON SEVEN
DIFFERENT COLONIES

Colony no.	No. Beagles	Age (yrs.)	Sex	PCV (%)	Eryth- rocytes (10 ⁶)	Hgb (g/100 ml)	Leukocytes (10 ³)	Neutro.	Band	Differential (abs.) [*]		
										Eosin.	Lympho.	Mono.
1	23	2-3	Both	...	7.76	17.4	10.8	6.0	0.1	0.5	2.3	0.5
2	46	1-9	M	47.6	...	16.0	14.2	8.2	0.3	1.0	4.1	0.6
2	68	1-9	F	46.8	...	15.6	14.8	8.5	0.3	0.8	4.7	0.6
3	51	1½-3	Both	51.6	7.50	16.7	11.3	7.7	...	0.3	4.2	0.8
4	37	1-3	Both	48.5	6.52	16.7	12.2	8.3	...	0.3	3.2	0.4
5	3	4	M	56.0	8.35	18.9	8.5	4.5	0.2	0.4	3.1	0.1
5	3	4	F	49.0	6.95	16.8	13.7	7.1	0.3	0.4	5.9	0.2
6	28	1-4	M	50.8	8.31	18.2	12.1	7.4	0.1	0.4	3.3	0.9
6	57	1-4	F	48.5	7.82	16.7	12.9	8.2	0.0	0.6	3.5	0.6
7	51	1½	M	51.6	6.94	17.2	12.9	8.6	0.5	0.5	2.9	0.3
7	47	1½	F	53.2	6.70	16.8	12.1	7.9	0.4	0.6	2.9	0.3

* Differentials expressed to the nearest 100, hence the sum may not equal the total leukocyte count.

TABLE XIX

Haematology values obtained at 4-5 months of age (Beagles)

Parameter	No.	Mean	(95% confidence limits)	Units
Hb	2723	12.5	(10.2-14.9)	g%
PCV	2723	40	(30-50)	% red cells
RBC	2723	4.83	(3.78-5.87)	$\times 10^6$ cells/cu. mm
MCHC	2723	31	(27-35)	%
MCV	2723	83	(66-100)	cu. microns
WBC (Total)	2723	15.6	(6.5-24.7)	$\times 10^3$ cells cu. mm
WBC (Neutrophils)	2723	9.3	(2.1-16.5)	$\times 10^3$ cells cu. mm
WBC (Lymph.)	2723	5.6	(1.4- 9.9)	$\times 10^3$ cells cu. mm
Platelets	2145	248	(103-392)	$\times 10^3$ cells cu. mm

Note: WBC and platelets vary little with age.

TABLE XX

Age-related changes of values relating to red cells (Beagles)

Parameter	Age (months)	No. of dogs	Mean	(95% confidence limits)	Method
Hb (g%)	4-5	2723	12.6	(10.2-14.9)	Cyan-metHb
	+ 1	256	13.7	(11.1-16.4)	(Cannon, R. K., <i>Amer. J. clin. Path.</i> , 1958, 30, 211)
	+ 3	396	14.6	(9.9-19.3)	
	+ 6	317	15.9	(13.2-18.5)	
	+12	220	16.4	(12.6-20.3)	
PCV (%)	4-5	2723	39.8	(29.8-49.9)	Haematocrit (Wintrobe)
	+ 1	256	42.7	(35.5-49.8)	
	+ 3	396	45.6	(38.0-53.2)	
	+ 6	317	49.0	(41.5-56.4)	
	+12	221	49.7	(42.1-57.3)	
RBC (x 10 ⁶ cells/cu. mm)	4-5	2723	4.83	(3.78-5.87)	Coulter Counter
	+ 1	256	5.21	(4.2-6.2)	
	+ 3	396	5.38	(4.3-6.5)	
	+ 6	317	5.81	(4.7-6.9)	
	+12	221	5.84	(4.8-6.9)	
MCHC (%)	4-5	2723	31.3	(27.4-35.2)	$\frac{\text{Hb (g\%)} \times 100}{\text{PCV (\%)}}$
	+ 1	256	31.8	(28.0-35.7)	
	+ 3	396	31.6	(28.5-34.8)	
	+ 6	317	32.2	(28.8-35.6)	
	+12	221	32.9	(28.0-37.9)	
MCV (cu. μ)	4-5	2723	82.9	(66.0-99.8)	$\frac{\text{PCV (\%)} \times 10}{\text{RBC (x 10}^6\text{/cu. mm)}}$
	+ 1	256	82.1	(68.8-95.4)	
	+ 3	396	85.0	(71.3-98.7)	
	+ 6	309	83.9	(71.9-95.9)	
	+12	221	85.2	(71.7-98.6)	

TABLE XXI

BLOOD SERUM VALUES FROM COLONIES OF BEAGLES
MAINTAINED AT THE UNIVERSITY OF CALIFORNIA
AT DAVIS (UCD) AND AT THE UNIVERSITY OF
ROCHESTER *

Constituent	UCD			U. of Rochester
Protein values				
Total protein (g/100 ml)	6.7 ± 1.1	(957)	5.9 ± 0.7	
Albumin (g/100 ml)	3.4 ± 0.4	(957)	2.7 ± 0.4	
Albumin %	51 ± 6	(1055)		
Alpha-1 globulin %	5.0 ± 2.0	(1055)		
Alpha-2 globulin %	11.2 ± 2.7	(1055)		
Beta-1 globulin %	12.9 ± 4.0	(681)		
Beta-2 globulin %	13.2 ± 3.3	(681)		
Gamma globulin %	6.7 ± 1.1	(1055)		
A/G ratio	1.04 ± 0.12	(1055)	0.86 ± 0.18	
Nonprotein nitrogen, carbohydrates, lipids, and pigments				
Urea nitrogen (mg/100 ml)	15.6 ± 7.1	(937)	12.2 ± 4.0	
Creatinine (mg/100 ml)	.81 ± .39	(101)		
Uric acid (mg/100 ml)	.67 ± .42	(731)		
Glucose (mg/100 ml)	99 ± 22	(931)		
Cholesterol (mg/100 ml)	218 ± 65	(936)		
Bilirubin (mg/100 ml)	.19 ± .20	(775)		
Inorganic constituents				
CO ₂ (meq/liter)			12.2 ± 4.0	
Chloride (meq/liter)	107 ± 9	(916)	110 ± 2.4	
Sodium (meq/liter)	182 ± 14	(69)	147.3 ± 3.1	
Potassium (meq/liter)	4.7 ± 0.5	(65)	4.7 ± 0.3	
Calcium (mg/100 ml)	9.9 ± 1.6	(775)		
Inorganic phosphorus (mg/100 ml)	5.4 ± 1.8	(852)		
Serum enzymes				
Serum glutamic oxalacetic transaminase (SGOT) Sigma frankel U/100 ml	34.0 ± 14.9	(279)*	27.8 ± 9.5 (Karmen units)	
Serum glutamic pyruvic transaminase (SGPT) Sigma frankel U/100 ml	27.2 ± 14.9	(273)		
Amylase somogyi U/100 ml	614 ± 110	(246)		
Alkaline phosphatase Bodansky U/100 ml	3.25 ± 2.17	(183)		

* Expressed as the mean ± S.D., number of samples in parentheses. Age ranges: UCD—120 to 720 days (293 dogs); U. of Rochester—½ to 7 years (63 dogs) for each measurement recorded.

TABLE XXII

Changes in Serum Chemistry Values for Young Normal Beagles

Constituent	Age in months ($\pm$ 10 days)	Number of samples	Mean	Standard deviation	Coefficient of variation (%)
Total protein (g/100 ml)	4	293	6.3	1.0	17
	8	195	6.5	0.9	13
	12	187	7.1	0.9	12
Albumin (%)	4	285	55	6	10
	8	192	51	5	9
	12	201	49	5	11
Alpha-1 globulin (%)	4	285	6	2	37
	8	192	5	1	19
	12	201	4	1	28
Alpha-2 globulin (%)	4	285	10	3	28
	8	192	13	3	28
	12	201	13	2	20
Beta-1 globulin (%)	4	104	13	4	28
	8	95	12	4	32
	12	148	13	3	27
Beta-2 globulin (%)	4	104	12	3	25
	8	95	13	3	20
	12	148	14	2	17
Gamma globulin (%)	4	285	5	2	48
	8	192	5	2	30
	12	201	7	3	40
Blood urea nitrogen (BUN) (mg/100 ml)	4	282	14	8.4	59
	8	197	17	4.6	27
	12	188	18	6.2	34
Uric acid (mg/100 ml)	4	166	0.74	0.51	68
	8	127	0.68	0.39	58
	12	181	0.63	0.36	57
Glucose (mg/100 ml)	4	287	108	20	19
	8	192	94	19	21
	12	186	95	17	18
Calcium (mg/100 ml)	4	245	10.4	1.5	14
	8	162	10.4	1.8	17
	12	134	9.4	1.6	17
Inorganic phosphorus (mg/100 ml)	4	213	7.2	1.8	24
	8	178	5.1	1.2	24
	12	185	4.4	1.0	23
SGOT (Sigma frankel U/100 ml)	4	70	27	10	36
	12	65	32	11	35
SGPT (Sigma frankel U/100 ml)	4	70	28	15	52
	12	65	21	9	41
Amylase (Somogyi U/100 ml)	4	70	560	90	16
	12	50	710	84	12
Alkaline phosphatase (Bodansky U/100 ml)	4	30	4.7	2.6	56
	12	53	2.2	0.9	41

TABLE XXIII

Normal biochemical values: Comparison between reported levels and H.R.C. levels

Parameter	Man ¹	Dog ²	Beagles (4-5 months old)			Units
			Mean	(95% confidence limits)	No. of dogs	
Urea	14-40	21-42	22.3	(11-33)	2692	mg %
Total reducing substances			102.7	(85-120)	1732	mg %
Glucose	60-95	60-100	94.7	(82-107)	897	mg %
SGPT	5-35	10-40	23.3	(11-35)	2520	SF units
SGOT	5-40	10-40	30.0	(14-46)	770	SF units
ICD			16.4	(6-26)	616	B & B units
Na	133-148	137-149	137	(125-149)	1225	mEq/l
K	3.5-5.5	3.7-5.8 (plasma)	4.6	(4.0-5.2)	1225	mEq/l
SAP	3-12 (adults)	7-15	20.4	(7-34)	2526	KA units
Proteins						
Total	6.5-8.2	5-7	5.3	(4.9-5.8)	2650	g %
Albumin	2.8-5.0	2.7-3.7	2.6	(1.9-3.3)	2650	
α_1 -globulin	0.15-0.45	0.7-1.0	0.42	(0.19-0.65)	2622	g % (electrophoresis)
α_2 -globulin	0.4-0.8		0.61	(0.32-0.91)	2622	
β -globulin	0.65-1.15	1.0-1.4	1.15	(0.89-1.41)	2650	
γ -globulin	0.8-1.6	0.6-0.9	0.56	(0.15-0.97)	2650	

¹ *Clinical Chemistry in Practical Medicine* (Stewart and Dunlop, 1962).² *The blood chemistry of the dog and cat* (Bloom, F.).

TABLE XXIV

*Serum alkaline phosphatase levels related to age of Beagles
(King-Armstrong units)*

No. of dogs	Age (months)	Mean (K.-A. units)	95% confidence limits
2526	4-5	20.4	7-34
273	+ 1	16.8	7-27
367	+ 3	14.2	7-22
311	+ 6	9.9	4-16
249	+12	10.6	4-17

TABLE XXV

*Comparison of laboratory results between some breeds of dog
(at 14-16 weeks of age)*

Test	Pembroke Corgi	Beagle	Cocker Spaniel	Units
	Mean (No./SD)	Mean (No./SD)	Mean (No./SD)	
Hb	12.2 (548/1.08)	12.1 (220/2.01)	-	g%
PCV	40.2 (1194/4.84)	39.6 (555/4.08)	37.0 (228/2.82)	%
RBC	4.87 (1154/0.687)	5.04 (555/0.571)	4.42 (228/0.492)	x 10 ⁶ cells/cu. mm
WBC Total:	16.8 (1194/5.28)	18.0 (555/5.83)	18.0 (228/4.84)	x 10 ³ cells/cu. mm
Neutrophils	11.0 (1005/4.31)	11.1 (555/4.67)	12.7 (228/4.44)	x 10 ³ cells/cu. mm
Lymphocytes	5.0 (1005/2.08)	6.1 (555/2.30)	4.7 (228/1.26)	x 10 ³ cells/cu. mm
Platelets	261 (485/82.8)	248 (288/75.4)	235 (63/71.3)	x 10 ³ cells/cu. mm
Urea	25.0 (991/6.94)	23.4 (554/6.43)	25.8 (228/8.54)	mg%
Total reducing substances	92.1 (921/14.22)	104.6 (503/8.34)	101.4 (228/9.61)	mg%
Alkaline phosphatase	14.1 (837/4.31)	18.5 (400/5.67)	18.4 (228/4.99)	King-Armstrong
SGPT	19.6 (546/4.82)	17.7 (220/7.38)	-	S.F.
ICD	20.1 (311/5.61)	18.0 (159/4.97)	22.3 (204/4.83)	Bell and Baron

TABLE XXVI

Internal Diameters of Bronchi in a 10-kg Beagle

	Diameter (mm)	
	Dorsoventral	Lateral
Right primary bronchus	12	13
Right apical lobar bronchus	7	6
Right cardiac lobar bronchus	4	3
Right secondary bronchus	6	7
Right diaphragmatic lobar bronchus	5	6
Right intermediate lobar bronchus	3	3
Left primary bronchus	8	10
Left secondary bronchus	5	5
Left apical lobar bronchus	4	4
Left cardiac lobar bronchus	3	3
Left diaphragmatic lobar bronchus	6	6

TABLE XXVII

Lung weights in Healthy Adult Beagles

Number of Beagles	Sex	Ref.	Body weight (kg) mean (range)	Lung weight (g) mean (range)	Relative lung weight* mean (range)
20†	M	(authors)	11.5 ± 1.0 (7.0-14.9)	75 ± 8 (41-104)	6.5 ± 0.4 (5.20-8.18)
20†	F	(authors)	10.6 ± 0.7 (7.0-14.1)	66 ± 5 (47-99)	6.2 ± 0.3 (5.17-7.74)
10	M	(6)		(65-119)	(6.15-8.49)
11	F	(6)		(48-95)	(6.06-8.49)
21‡	M	(7)	11.0 ± 2.1		8.5 ± 1.5
50†	F	(7)	8.3 ± 0.6 (5.0-14.8)	60 ± 5 (38-105)	7.2 ± 0.3 (4.00-10.6)
22‡	F	(8)	8.4 ± 2.1		8.98 ± 1.5

* Grams per kilogram of body weight.

† Expressed as the mean and 95% confidence interval around the mean.

‡ Expressed as the standard deviation of the mean.

TABLE XXVIII

Relative Weights of the Right and
Left Lung and Individual Lobes*

	Percent of total lung weight	
	Male	Female
Right lung	56.7 $\pm$ 1.4	57.5 $\pm$ 0.6
Left lung	43.0 $\pm$ 1.3	42.5 $\pm$ 0.6
Lobes, right lung		
Apical	15.3 $\pm$ 1.8	15.5 $\pm$ 0.9
Cardiac	8.3 $\pm$ 1.2	9.3 $\pm$ 0.7
Diaphragmatic	24.9 $\pm$ 1.7	23.6 $\pm$ 2.6
Intermediate	8.3 $\pm$ 0.6	8.2 $\pm$ 0.9
Lobes, left lung†		
Apical	10.3 $\pm$ 0.7	9.6 $\pm$ 0.5
Cardiac	6.4 $\pm$ 0.9	6.4 $\pm$ 0.6
Diaphragmatic	26.9 $\pm$ 1.6	26.3 $\pm$ 0.6

* Results from 20 female and 10 male Beagles.

† Left apical and cardiac lobes were divided along the most apparent fissure line.

TABLE XXIX

Clearance Rate of Various-Size Particles in the Lower Respiratory Tract of the Beagle

Material	Particle size CMD/ σ g	Number of Beagles	Time interval (days)	Pulmonary compartment burden	Pulmonary clearance half time* (days)	Total deposited (μ g)	Ref.
²³⁹ Pu (on NaCl)	.04/2.30	6	28-149	...	36	...	19
⁵⁹ Fe ₂ O ₃	.09/1.78	6†	2-~30	.87‡	62	...	20
⁵⁹ Fe ₂ O ₃	.06/1.80	4	2-~50	.89‡	58	50	21
⁵⁹ Fe ₂ O ₃	.15/2.00	3	5-50 20-100	.20§	160-220	7,000	22
³ H ₂ O	.16/1.68	3	2-~40	.55‡	33	200	21
¹³⁷ BaSO ₄	.10/1.86	1	5-20	...	8	90	21
⁵⁴ MnO ₂	.07/1.66	2	2-~60	.68‡	34	80	21
¹³¹ I ₂ O ₅	.31/1.81	3	~10-80	.36‡	200	18,000	21
²³⁹ PuO ₂	.12/1.61	3	5-~33	.25-.80§	30	2	23
²³⁹ PuO ₂	.32/2.25	3	5-~33	.50-.70§	200-500	8	23
²³⁹ PuO ₂	.43/2.28	3	5-~33	.40-.60§	120-220	5	23
²³⁹ PuO ₂	.24/2.83	3	5-~33	.30-.60§	180-250	8	23
²³⁹ PuO ₂	.30/2.86	3	5-~33	.40-.60§	140-220	300	23
²³⁹ PuO ₂	.60/2.25	3	5-~33	.50-.85§	250	500	23
²³⁹ Pu(NO ₃) ₃	.13/2.24	3	5-~33	.40-.70§	40	300	23

* Corrected for radionuclide decay. † Two tests per Beagle. ‡ Fraction of initial lung burden.

§ Fraction of total deposited. CMD Count median diameter. σ g Standard Geometric Deviation.

TABLE XXX

Average Measurements of Adult Canine Brains

Breed	Number of dogs	Body weight 52-week age (kg)	Brain weight (g)	Brain width (cm)	Brain length (cm)	Brain volume (cc)
Shetland Sheepdog	9	60.31*	10.5	4.85	6.27	68
Cocker Spaniel	10	79.44	9.23	5.36	6.71	78
Wire Fox Terrier	4	68.66	7.7	4.86	6.5	70
Basenji	7	53.96	10.9	4.58	5.78	45
Beagle	9	72.75	10.6	5.07	6.45	72

* Greatest variations were found in this breed, weights ranging from 51.2 to 68.1 g.

TABLE XXXI

Reflexes in Neonate Dogs at Various Ages*			
Reflex responses	Strong	Weak, variable	Absent or adult-like
Crossed extensor reflex	1-16	16-18	18+ (absent)
Magnus reflex	1-17	17-21	21+ (absent)
Neck extension posture	Flexion 1-4	Hyperextension 4-21	Normotonia 21+
Forelimb placing	4+	2-4	0-2 (absent)
Hindlimb placing	8+	6-8	0-6 (absent)
Forelimb supporting	10+	6-10	0-6 (absent)
Hindlimb supporting	15+	11-15	0-11 (absent)
Standing on all fours	21+	18-21	1-18 (absent)
Body righting (cutaneous)	1+	0-1	...
Blink response to light	16+ days	4-16	0-4 (absent)
Visual orientation	25+ days	20-25	0-20 (absent)
Auditory startle reflex	24+ days	15-24	0-15 (absent)
Second orientation	25+ days	18-25	0-18 ...
Rooting reflex	0-14	14-25	25+ (absent)
Nociceptive withdrawal reflex	0-19	19-23	23+ (adult-like)
Panniculus reflex	0-19	19-25	25+ (adult-like)
Reflex urination	0-22	22-25	25+ (absent)

* Ages given in days.

TABLE XXXII

ENDOCRINE ORGAN WEIGHTS IN DOGS OF VARIOUS
BREEDS AND IN THE BEAGLE

Breed	Age (mo.)	Sex	Average body wt. (kg)	Thyroid		Adrenal		Hypophysis	
				Average absolute wt. (g)	Average relative wt.	Average absolute wt. (g)	Average relative wt.	Average absolute wt. (mg)	Average relative wt.
Miscellaneous (21% Beagles)	0.10-0.25	M,F	0.2-0.3	0.003	0.217	0.10	0.74		
	0.25-0.50		0.4-0.7	0.141	0.210	0.18	0.47		
	5.0-8.0		4.0-7.0	0.517	0.154	1.13	0.17		
	12		8.5-2.9	0.758	0.103	1.27	0.14		
	36-48		11.8	1.042	0.086	1.76	0.15		
	120.00		17.3-17.8	0.514	0.030	2.87	0.19		
	144-180		13.1-14.3	0.842	0.061	2.81	0.23		
Beagle	1.5-4.0	M,F	5.38±0.76	0.70±0.40		0.70±0.5			
	7.8-91.8		9.50±2.39	1.00±0.40		1.4±0.6			
	6.5-8.0	M			0.06-0.10		0.07-0.08		
	8.0-8.5	F			0.08		0.08-0.10		
	8-38	M		0.5-1.6	0.05-0.14	0.16-1.70	0.06-0.13	60-90	0.005-0.009
		F		0.4-1.3	0.04-0.13	0.70-1.90	0.08-0.18	48-103	0.006-0.009
		M,F		0.95-1.65	0.083-0.128				
36-48	M,F		8.79±1.62		0.103±0.020	rt. adrenal l. adrenal	0.084±0.014 0.085±0.015		
			12.7±2.8			0.96±0.14	0.077±0.011		

TABLE XXXIII

Probability of Survival of Female Beagles (control) Which Entered a Life-span Experiment at One Year of Age*

Age (months)	$P_x \pm S_x$	Mortality rate (%)
12 through 23	100 ± 0	0
24 through 35	97 ± 2	3.4
36 through 47	95 ± 2	1.8
48 through 59	93 ± 3	1.8
60 through 71	86 ± 4	7.3
72 through 83	81 ± 5	5.9
84 through 95	78 ± 5	4.2
96 through 107	76 ± 6	2.2
108 through 119	71 ± 6	6.7
120 through 131	61 ± 6	14.6
132 through 143	52 ± 7	14.7
144 through 155	46 ± 7	12.0
156 through 167	27 ± 7	40.0
168 through 180	12 ± 8	57.1

* Fifteen of the 60 Beagles alive when accumulated data were analyzed.

The Dalmation, once known as the Carriage Dog or Coach Dog, has not been widely employed in research, although its well-known biochemical abnormality, its characteristic spots and its inborn behavioural characteristics have led to its use in genetic studies.

Hubbard (1948) states that the origin of the breed, and its early history, are both obscure, but considers that it was once a type somewhere between a Pointer and the so-called Lesser Danish Dog. He refers to Buffon's description of a spotted dog of similar size called a 'Bengal Harrier' and to Taplin's belief that the Dalmation was a relation of the Great Dane, but concludes from his own investigations that it is probably descended from the Istrian Pointer, with the possible introduction at some stage of occasional crossing with a Great Dane to provide depth of muzzle and added tone of colour. The Istrian Pointer resembles the Dalmation but its markings are not in uniform spots nor so dark in colour. The spotting of Dalmations is almost certainly a form of the ticking referred to in 3:5. (Burns, 1952).

The Dalmation was once employed as a gun-dog in Italy, Austria and elsewhere and the first evidence of its use as a coach dog appears to date from about 1665. The head is

fairly long, rather broad between the ears and the skull is flat on top. The mouth is strong and level. The chest is deep and the ribs appear strong and somewhat prominent. The coat is dense, short and smooth, without being silky or woolly in texture. The height at the shoulder is generally from 50 to 58 cm and the adult weight is about 25 kg for the male and 20 to 23 kg for the female.

Benedict (1916) reported on a Dalmation that excreted almost as much uric acid per day in its urine as an adult man. When receiving a purine-free diet, it eliminated uric acid at a rate of 36 mg/kg bodyweight/day. This rate remained constant even with a large variation of from 2 to 24 g in the protein nitrogen (N) of the diet. He examined 4 others, 3 of which were found to have a similar pattern of uric acid excretion while the fourth proved an exception and was '... obviously not of very pure breed'. This suggestion that the breed differed from others in its purine metabolism prompted Wells (1918) to investigate the distribution of the relevant enzymes in the tissues of a Dalmation. Uricase, which assists the conversion of uric acid to allantoin, was present in the liver, so that the capacity for excreting uric acid could not be explained by its absence. Folin, Berglund & Derick (1924) and Krafka (1929, 1930) have confirmed the existence of high uric acid excretion in Dalmations representing strains of the breed widely separated geographically.

Onslow (1923a, b) investigated the heritability of uric acid excretion by hybrid descendents of a Dalmation dog. A terrier and some of its descendents excretes allantoin as the principal purine end-product, and their output of allantoin N was recorded as from 16 to 21% of the total N, whereas the uric acid N was only from 0.2 to 0.4% of the total, i.e. the uricolytic indices, i.e. $\frac{\text{allantoin N}}{\text{uric acid N} + \text{allantoin N}} \times 100$, were 98 to 99. The Dalmation ancestor and one of its female descendents excreted 2 to 3% of the total urinary N as uric acid N, while the allantoin N formed from 13 to 17%, i.e. the uricolytic indices were 84 to 85. Trimble & Keeler (1938) observed the purine excretion of a family of Dalmations, two Collies, and upon their hybrid descendents, all of which were fed upon purine-free diets while under observation, and found evidence that the high uric acid excretion was due to a recessive autosomal gene. At the same time, the inheritance of spotting was studied, and it seemed quite clear that there is not any linkage between the genes responsible for high uric acid excretion and for spotting, i.e. the characters are determined by genes in different chromosomes. Their numerous observations never yielded a uricolytic index higher than 58% for a pure-bred Dalmation, nor an allantoin output higher than 5% of the total N. In a subsequent study, Weatherford & Trimble (1940) reported that there were fewer intranuclear crystals in the hepatic cells than in other

breeds. The subject has been reviewed by Wagner & Mitchell (1964), while Harvey & Christensen (1964) have concluded from their investigations on the erythrocytes that the Dalmation has a defect in the transport system that prevents uric acid from reaching the interior of various cells. There is of course an inheritant danger in the Dalmation of urolithiasis associated with uric acid concretions.

Trimble & Keeler (1939) observed that when Dalmations are trained to follow a carriage there were distinct individual preferences in the position taken up. Some chose to run under the carriage, with their noses close to the horse's heels. Others preferred to run behind the carriage, while others again occupied intermediate positions. Each dog was found always to run in its preferred place, and this 'position preference' appeared to be familial, although it could not be explained in a simple Mendelian manner. Burns (1952) states that Dalmations are reported to have an inborn love of horses. (See also Weiss, 1940.)

Richards & Hoe (1967) included in their study of long-term renal disease in the dog the details of a Dalmation that, at the time of their publication, had already survived for a further 3 years since, at 6 years of age, it had manifested a blood urea level of up to 300 mg/100 ml.

Keeler & Trimble (1938) studied the inheritance of dew claws by means of Dalmation-Collie crosses.

Hubbard (1947, 1948) has provided accounts of the origins of the Collie, or Scottish Collie, the name of which derives from the term 'colley' given to the native mountain sheep from which black-faced sheep are descended. The name 'Colley Dog' or 'Scotch Colley Dog' is now obsolete, but the Scottish Collie is a well recognized sheep dog of the present day. There are several varieties, including the Bearded Collie or Highland Collie (once termed the Hairy Mountain Collie) and the Smooth-Coated Collie, and the height at the shoulder varies from about 48 to 58 cm, while bodyweight is of the order of from 23 kg for females to over 27 kg for males. Working specimens have a strong muzzle and the long, lean and clean-cut head, moderately wide between the ears and flat on top, is characteristic of the breed. The eyes are brown except for the blue-and-white 'wall eyes' seen in merle dogs. The body is fairly long, with deep chest and well sprung ribs, and the legs are straight. In the rough-coated varieties there is a profuse growth of hair, the outer ones being quite harsh.

A much smaller breed, the Shetland Collie or Shetland Sheepdog, is almost a miniature of the Scottish Collie, although it has a relatively narrower head, more compact body and shorter back. The height at the shoulder is not, for show purposes, supposed to exceed more than about 36 cm, while the 'ideal' bodyweight is given as under 6.5 kg.

Worden, Noel & Jolly (1963) suggested on the experience of one of the authors that the Collie was not well suited to research, since they did not take kindly to a cloistered existence or to strangers, and tended to be aggressive towards other dogs.

Lulling and his co-workers (Lulling, Lievens, El Sayed & Prignot, 1968; Lulling, Prignot & Lievens, 1968; Prignot & Lulling, 1969) and also Giurgea (1971) have employed the Collie successfully for studies on experimental bronchopneumopathy produced by exposure to sulphur dioxide and for the investigation of bronchodilators, although they found that there were fewer bronchial glands than in man and that they were irregularly distributed, so that it was not possible to assess the pathogenesis of bronchitis by repeated bronchial biopsies. In their investigations on bronchopneumopathy, the animals were shaved to facilitate cutaneous thermoregulation and to avoid thermal hyperventilation during functional examinations. They recorded bronchial disease, often severe, and were able to confirm this by bronchoscopy and bronchography. They noted, however, fairly discrete clinical and radiological evidence of spontaneously resolving broncho-pneumonia in almost all their animals at one time or another. They never observed, even after repeated exposures to sulphur dioxide, a glandular hypertrophy comparable to that described in bronchitic men or rats (Reid, 1963), and stated that the histopathological appearance

resembled that described by Rossing (1964) in his studies on exposure of dogs to phosgene, with a bronchiolitis obliterans in animals surviving to the 4th week, often associated with atelectasis and at other times with hyperdistention of the air spaces. The hypersecretion was ascribed to a combination of chemical irritation and bacterial infection. Whereas there is an abundance (up to 77% of the dialysable fraction) of mucins in human bronchial secretions, only 10% was recorded in the bronchitic dogs, while electrophoretic and immunological studies confirmed that in the latter species the secretion was essentially purulent. Nevertheless Lulling and his co-workers concluded that the persistent bronchial hypersecretion and the functional obstructive syndrome observed in the Collie following exposure to sulphur dioxide had a clinical, endoscopic and functional symptomatology closely resembling that seen in bronchitic man, and that the breed was therefore useful for experimental studies of this kind.

Kelley (1947) showed that linebreeding could fix outstanding working qualities in a strain of 'Trial-bred' Border Collies, but attributed the inheritance of 'eye' to the control of multiple genes. He reported that ease of learning was at least sufficiently inherited to make selective breeding for it effective. Kelley's observations on coat colour indicate how long a recessive condition may lie hidden until 'discovered by suitable gene combinations', and he attributed dew-claws on the hind legs to a recessive

condition. Keeler & Trimble (1938) had reported variable dominance of dew-claws in Dalmation X Collie crosses. Kelley reported the occurrence of 3 Border Collie puppies with shortened lower jaws among 12 offspring of a dog and his 2 half-sisters, all the parents being normal, which again suggested a recessive mode of inheritance. A form of deafness in this breed, which did not become noticeable until about 2 years of age, seemed to be due to a single recessive factor and was not associated with white coat colour.

In a study of a single outbreak of viral hepatitis, involving 38 dogs, Parry (1950) recorded breed differences in response. Of 4 Border Collies at risk, 3 developed the disease, but none died.

Renton & Aughey (1971) have reported on an infertility of unknown aetiology in 2 Shetland Collie males with earlier records as proven sires. Clinical examination did not reveal any obvious abnormalities, and libido was excellent, but spermatozoa were absent from the semen collected with the aid of an artificial vagina. Testicular biopsy revealed that the seminiferous tubules were lined by spermatogonia and Sertoli cells with occasional areas where spermatogenesis has proceeded to the primary spermatocyte stage, while the lumina of the tubules were filled with an amorphous material.

Reference has been made in 3:4 to the inheritance of umbilical hernia in Collies, as in Cocker Spaniels and Bull Terriers.

As noted for other breeds, Collies that are heterozygous for the dominant factor known by the symbol M, which is responsible for merle coat colour often have 'wall eyes'. Two merles that are mated together have a proportion of white or almost white offspring, with various abnormalities, including deafness, blindness and structurally abnormal eyes.

Heape (1908) recorded an average litter size for 'Collies and Sheepdogs' of 7.3 g, with a sex ratio of 118.19 males: 100 females, but Kelley (1951), for Border Collies, has corresponding values of 5.7 and of 84.9:100.

The average measurements of Shetland Sheepdog brains are included in Table XXX.

The Basenji appears to represent a breed or type that has been identifiable for some thousands of years. Hubbard (1948) states that Egyptian rock-carvings of Dynasty V (circa 2700-2600 B.C.) depict dogs that were markedly like the present-day Basenji, and that the breed was undoubtedly present at one time in Upper Egypt, if not in Lower Egypt. The introduction of the animal into Western Europe and the United States was, however, a modern occurrence, the first importations into Britain being in 1934, and the first exhibition of specimens in 1937. A separate register was granted by the Kennel Club in 1941, and a show, under the auspices of the Basenji Club of Great Britain, was held in 1946.

These importations derive from the inner Congo basin, and synonyms for the breed include Belgian Congo Dog, Congo Bush Dog and Congo Hunting Terrier, while there appear to be closely related types in central Africa, the Sudan and the Upper Nile, e.g. the Niam Niam and the Manboutou. As bush dogs the Basenji and its near-relatives are used for hunting antelope and for beating game. Hubbard states that: 'Usually a "king" dog is selected to lead the beaters, working with a dried gourd filled with pebbles (or a bell) tied around its neck to make the greatest noise, for it must be borne in mind that the Basenji is practically mute'.

It is of course the lack of a normal bark that has interested research workers, but there is vocalization, sometimes referred to as crooning or yodelling, which Worden, Noel & Jolly (1963) suggest may be equally irritating. Scott & Fuller (1965) reported studies on Basenji X Cocker Spaniel crosses, and from their observations on the F_1 and F_2 generations and on the backcrosses they found evidence of a single dominant gene responsible for being 'easily stimulated to bark'. These same studies helped, incidentally, to establish the dominance of short hair over long hair in the dog. Burns (1952), in her listing of the distribution of the two types of yellow-red coat colour between breeds, includes the Basenji as among those having the Agouti series, Yellow, Sable, a^Y only.

When first brought to Britain the female Basenjis came into oestrus only once a year, but according to Burns (1952) 'heats are now occurring at intervals of six to eight months in many bitches in this country, as is the general rule in other breeds.'

The behavioural studies of Scott and his co-workers (Scott & Marston, 1950; Scott & Fuller, 1951; Fuller, 1953; Scott, 1958; see also Worden, 1959) have provided a clear indication that the Basenji is among the types of dog that are much shyer at 5 weeks of age than, e.g., the Cocker Spaniel, and that this difference is inherited on Mendelian lines, with probably two factors involved. As already

indicated in 3:6, Basenjis were more sensitive to restrictive handling than Beagles but, like Cocker Spaniels and Wire-haired Fox Terriers, were more skilful than Beagles in employing motor skills, climbing boxes and walking a plank in order to obtain food (Scott & Fuller, 1965). Reference to Beagle X Basenji crosses, and their possible use as an experimental animal, produced by Dr. M.K. Abelseth of the Cannaught Medical Research Laboratory, University of Toronto (see Andersen, 1970, p.7) has also been made in 3:6. These hybrids were quieter than Beagles but not barkless. So far as the present author is aware, however, the experimental use of the Basenji has so far been confined almost entirely to genetic and behavioural studies, and there is no evidence that it has received serious attention as a routine participant in long-term nutritional or toxicological investigations.

Hubbard (1948) indicated a shoulder height of about 56 cm and a bodyweight of about 9 to 10 kg. He drew attention to the cleanliness of the breed, stating that it was 'known to wash itself with licked paws like the cat'.

The average measurements of Basenji brains are included in Table XXX.

The term 'Husky' - synonymous with Eskimo - is applied to a considerable number of types of dog, some of which qualify as distinct, if sometimes related, breeds and varieties. Hubbard (1947) has pointed out that the group of dogs referred to derives from localities that extend from Alaska and the Yukon through the Mackenzie River basin of Arctic Canada, the Hudson Bay surrounds, Baffinland and Labrador through to East Greenland. He states that, broadly speaking, '... dogs hitched to sleds say below about the 55th parallel are to a very large extent either Huskies showing a considerable infusion of alien blood or dogs which are not in the least like the true Husky at all. North of this line the quality of the dogs improves progressively, and more closely approaches the true type, showing every evidence of meticulous breeding..... Hence we find in Alaska a fine race of sled dog bred by the Malamute natives and named after the tribe; in the Yukon basin a large lupine type is prevalent, known as the Timber-wolf Dog; in Baffinland there exists a local type only slightly differing from the Husky proper; in Labrador another breed has long been extant; and in Greenland a good type is used on the west coast, whilst an even older breed exists on the eastern coastline..... The term is also used in a specific sense as meaning the sled dog most widely employed in the

Canadian backwoods, particularly in the North-West Territories. The dog of these areas is the Husky proper, the breed of which has been officially recognized under the title of Eskimo by the American Kennel Club. The true Husky is a fairly square-built dog admirably designed for heavy work. Its short neck and cobby build and curled tail denote an increasingly distant removal from the blood of the wolf. There are [however] many accounts which show that the Husky will breed freely with the timber-wolf and that many of the Indians purposely allow their bitches to run in season quite loose when these wolves are about. . . . The Timber-wolf Dog, for instance, is quite half-wolf; the breed is, moreover, in great demand as leader in a team, for it has much intelligence and sets good example in its work.' Hubbard goes on to refer to the significance of tail carriage, which is low to the ground or typically lupine in the Timber-wolf Dog but well on the back in the true Husky, and to the disparity between sheer size and actual performance as a sledge dog.

The East Greenland Husky, known as the Angmagssalik Husky or Østgrønlands Hund also, is probably the purest breed of sledge dog to be found in the Western Hemisphere, and seems to show more affinity with the Siberian Chuchi than with other Western dogs. It is a well-built, muscular and compact dog, with average shoulder height of about 58 cm and a bodyweight of approximately 30 kg. Many, but not all,

sledge dogs employed for Expeditions have been of this breed.

Tinbergen's (1942) observations have been summarized by him (Tinbergen, 1951; see also Worden, 1959) as follows: 'The Eskimo dogs of East Greenland live on packs of 5-10 individuals. The members of a pack defend their group territory against all other dogs. All dogs of an Eskimo settlement have an exact and detailed knowledge of the topography of the territories of other packs; they know where attacks from other packs must be feared. Immature dogs do not defend the territory. Moreover, they often roam through the whole settlement very often trespassing into other territories, where they are promptly chased. In spite of these frequent attacks, during which they may be severely treated, they do not learn the territories' topography and for the observer their stupidity in this respect is amazing. While the young dogs are growing sexually mature, however, they begin to learn the other territories and within a week their trespassing adventures are over. In two male dogs the first copulation, the first defence of territory, and the first avoidance of strange territory all occurred within one week.' Worden (1959) has described the reactions of a growing Husky pup to a Scottish Terrier pup of its own age with which it was brought up and which it was at first dominated by, but later learned to dominate, and to an older Scottish Terrier that it failed to recognize as such, and towards which it made a typical gesture of surrender, lying supine with its neck exposed.

Adie (1953) recorded the successful prevention of unwanted matings in a group of Huskies by the use of a proprietary preparation based on essential oils (see 4:2 and also Worden, 1939).

The use of the Husky as an experimental animal, except for breeding or behavioural observations such as those of Krusinskii (1946), who included the Eskimo dog in his attempted correlation between constitution and behaviour within various breeds and types, has related to Expeditions. The problem of providing a suitable food for the long sledging journeys in Arctic or Antarctic conditions has long been evident, for the greatest proportion of the initial load on the sledges is made up of dog diet, the 'fuel' for the journey. These studies on the nutrition and physiological performance have not been without their predictive value for human explorers, while they have provided some interesting veterinary and biochemical information.

Taylor (1957c) discussed the breeding and maintenance of sledge dogs in the Antarctic and Taylor, Worden & Waterhouse (1959) produced a diet that was more satisfactory than the dog pemmican that had been in use for Expeditions over the previous 30 years in the Antarctic and in North Greenland (see Watkins, 1932; Lewis, Masterton & Ward, 1957; Masterton, Lewis & Widdowson, 1957), and drew attention to the need to reinvigorate the dogs by occasional meals of fresh seal-meat.

The maximum work output which could be maintained over 10 minutes by a Husky weighing 39 kg was found by Taylor (1957b) to be 0.235 horse-power, which on a kg bodyweight basis was very close to the maximum recorded for man. Maximum work output was similar for walking and for trotting.

Taylor et al. (1959) recorded that on the hitherto standard daily ration of 1 lb. pemmican, a dog would lose weight even when not working. During sledging the faecal dry matter was mostly protein, and faecal fat did not exceed 7% on a dry-matter basis, compared with the 23% total fat in the faeces of men on Expedition (Masterton, Lewis & Widdowson, 1957). Fat utilization from pemmican appeared therefore to be reasonably good, but there was evidence of much protein wastage, shown not only by the high rate of faecal loss but also by the development of an indicanuria (see 4:7). Another apparent abnormal result from pemmican feeding was the appearance of a fluorescent pigment in the urine, even in dogs maintained in metabolism cages at HRC, while under these conditions there appeared to be water-retention over the study periods of up to 5 days, but no oedema or gain in bodyweight. Indeed, there were sometimes loose stools and a fall in bodyweight. The fluorescent material, which was not thiochrome, interfered with determination of vitamin B₁ or thiamine. Vitamin A excretion appeared to be higher on

pemmican than on their standard control ration based upon rabbit flesh, wholemeal bread and cow's milk, with various supplements, but the values recorded were not significantly outside the range reported for adult dogs of other breeds by Worden, Bunyan, Davies & Waterhouse (1955), ranging from 67 to 214 International Units/24 hours.

Taylor et al. devised what they recorded as a more suitable diet comprising white-fish meal, skim-milk powder, debittered dried yeast, precooked maize starch, margarine, beef suet, ethyl gallate and various vitamins. The new diet and pemmican were given on alternate days to all dogs on a 250-mile traverse in the Shackleton Mountains and during the journey of 570 miles between South Ice and the South Pole (see Fuchs & Hilary, 1958). 'The huskies had diarrhoea after each feed of pemmican, but not after the new diet on which, in the opinion of their drivers, they did far better..... Faeces excreted on the new diet contained 4.2% nitrogen and 14.6% fat, compared with 11.0% nitrogen and 5.5% fat on pemmican.' (See also Orr, 1965).

It is a pleasure to record with grateful thanks not only the help and inspiration given by Sir Vivian Fuchs to the work undertaken prior to the Commonwealth Trans-Antarctic Expedition, but also his subsequent gift to the present author of a lead-dog, 'Bouncer' which crossed from South Ice to the South Pole.

4 : THE USE OF THE DOG AS AN EXPERIMENTAL ANIMAL

4 : 1 Breeding, selection and preparation of animals for experiment

Dog-breeding has its own extensive semi-popular literature, some aspects of which have been referred to in Chapter 3, and actual breeding practices have been reviewed and summarized by Whitney (1949) and others, including Kelley (1947), whose discussion of sheep dogs applies to a considerable extent to other breeds. Harrap (1960) produced a standard text on reproduction in the species, while Hancock & Rowlands (1949) has earlier reviewed its reproductive physiology (see also the detailed information in the Beagle already referred to in 3:6) and Burns (1952) its genetics. More recently Paterson (1967) has dealt with the breeding of laboratory dogs generally, and Appleton & Appleton (1970) and Andersen (1970, pp.31-39) with the breeding of the Beagle.

The practical experience of the author and of his colleagues at HRC itself has been limited largely to experimental breeding studies, including those in which it has been deemed desirable to include the dog among the species to be employed for teratogenicity testing. All dogs employed for research otherwise are bought in from a single source, the Appleton & Appleton kennels, which currently produce some

2,500 young Beagles a year for HRC alone and up to a total of 6,000 Beagles a year for research workers generally. The animals received at HRC are produced either in the Appleton & Appleton's own kennels, now transferred to Wales, or with the aid of their satellite units, but in all instances the complete pedigree is available. The degree of control over source material is of course less than in the case of rats and mice, both of which are bred in extensive numbers on site in controlled environmental conditions, but is in marked contrast to that operating in the case of primates (Heywood, 1969). The close association with a single breeding organization, with the detailed records maintained, does permit of a check upon spontaneous conditions and enable those that are not widely distributed genetically to be 'bred out'.

The author's colleague Noel (1969) calculated that a purebred dog purchased at the age of 14-16 weeks cost approximately £30.00, compared with £1.00 for an 'SPF' rat aged 4-5 weeks, £12.25 for a pure-bred pig aged 8 weeks and £30 for a baboon or rhesus monkey aged 1 to 2 years and hitherto maintained in isolation for 8 weeks after capture in the jungle and transport to the U.K.

On arrival at HRC, a 4 week predosing or pre-feeding is normally allowed - for some nutritional studies the dogs may be required at a younger age and subjected earlier to an experimental routine - and during this time the animals are subjected to repeated veterinary clinical examination,

including ophthalmoscopy and electrocardiography, as discussed in 4:5. They are reinoculated against canine distemper, canine hepatitis or 'Rubarth's disease', and leptospirosis, and are given a course of an anthelmintic. Prothrombin times are estimated to check for the existence of Factor VII deficiency, and the haematological and biochemical determinations described in 4:6, and checks upon urinary specimens as described in 4:7, are made. The animals are weighed upon arrival, at which time they are ear-marked, and at weekly intervals thereafter, so that experimental groups may be constructed upon the basis of age, sex, litter of origin and bodyweight. Any individual that seemed to be temperamentally unsuited would be eliminated during this period, as of course would any animal showing evidence of some detectable biochemical or other abnormality, or of a disease condition.

'The minimum essential requirements, whether for breeding or experimental observations, are protection from the weather, a dry, comfortable bed, and adequate space for exercise' (Paterson, 1967). 'No one kennel design can meet all experimental requirements because location, economics, and researcher demands are extremely variable. Although the Beagle can adjust to a wide range of environments, certain features in a kennel design may cause undesirable effects. For example, confinement has been shown to cause stereotyped behaviour [Fox, 1965], higher (5 percent) pup mortality [Andersen, McKelvie & Phemister, 1962], and variation in muscular performance Yoder, Kingrey & Dragsteat, 1964Cages should only be used to maintain Beagles for a limited period of time except when exercise runs are provided. In general, maintaining a large colony exclusively in cages indicates maximum confinement, a situation favoring the spread of disease and parasites.... Most kennels using cages have a common exercise area, and such areas serve as a disease reservoir as well as an increased work load' (Andersen, 1970, pp.10-19).

Cages are not favoured for experimental use in the U.K., but an adequate description of them as employed in America is provided by Andersen (loc.cit). In the U.K., kennelling is often of the outdoor-indoor type, as described by

Paterson (1967). At HRC this type has been replaced by indoor runs with separate outside exercising areas, briefly described by Noel (1969) as follows: 'Our dogs are housed in internal kennels, six feet long by two feet wide with separate outside areas allocated for exercise. The best kennels can be turned into metabolism cages by the addition of trays and moveable partitions, thus causing little disturbance when urine and faecal samples have to be collected. It is considered essential that kennels to be used for toxicity studies should have under-floor heating. When severe signs result in disturbance of the central nervous system, adequate warmth is necessary to counteract any tendency for pneumonia to develop'. Some of the indoor runs at HRC are somewhat larger, and some may be converted easily by removal of a partition to become approximately 183 cm x 122 cm to house 2 or 3 animals.

'Sawdust is sprinkled onto the solid floor, thereby allowing the animal to remain clean: the risk of skin lesions is reduced, and the urine is absorbed. Each run is cleaned once daily... The runs or kennels are arranged down each side of a room, with doors opening onto a wide central passageway, and with the daily records affixed to the other side of the door in much the same way as a patient's records are hung at the end of his bed. The dogs are, in fact, housed in a ward system, with a separate room at the end of each ward to house the person who is responsible for the maintenance, weighing, dosing and recording of the animals

in that particular ward - usually constituting one experiment and sometimes two Special examination and service rooms are at one end of the line of wards, separated by a corridor, while there is a passage for the delivery of food, and the removal of sawdust and excreta at the other end. Beyond that are the exercise pens, which are in the open air. For each ward there is a relatively large exercise pen into which animals are allowed to run as a group, under strict supervision, and there are in addition two smaller pens into which one or more animals may be removed for individual study. The behaviour of the animals during exercise, especially when in a group, may perhaps be the first detected change to indicate a drug response' (Worden, 1970, pp.62-63). Each outside exercising pen measures approximately 6 x 6 m, and the smaller individual pens opening off it approximately 1.5 m x 1.5 m.

The general management of experimental dogs has been described by the U.S. Department of Health, Education and Welfare (1965), Paterson (1967) and McKelvie (1970). The most important considerations apart from good kennelling and sound feeding practices (see 4:3) and adequate disease control (see also 4:9) are to have sympathetic and well-trained attendants. In many countries it is the practice to leave actual animal care to what are described in the U.S.A. as animal caretakers, and indeed it would appear to be contrary to the trades union practices in certain States to entrust to the same persons the actual care of the animal

and the carrying out of even routine experimental procedures such as the withdrawal of blood samples. At HRC, the author and his colleagues are fortunate in having considerable numbers of research assistants, all or almost all of whom are studying for, or have passed, the examinations of the Institute of Animal Technicians (see Porter & Hill, 1967) and are trained to observe as well as to care and to be capable of assisting significantly with the carrying out of routine observations and routine experimental procedures. They in turn are responsible to research officers, mainly science graduates who have spent several years working with dogs or other species and who support the veterinarians, medical men and other senior scientists who devise or interpret the experiments.

There have been numerous, often vigorous and rarely agreed discussions between those who favour the maintenance of dogs in completely, or almost completely, controlled environments and those who prefer not only outside exercising pens but also access to earth, or grass, paddocks as opposed to concrete runs. At HRC it has been the practice-- in non-breeding dogs - to confine outdoor exercise to concrete floor pens, although in earlier days, when the experiments were purely of a nutritional nature and the numbers of dogs were few, the animals were actually taken for walks on nearby common land.

One of the problems associated with access to grass

has been indicated by Walker (1971). As he points out, grass provides a source of nutrients other than the food given, including β -carotene, vegetable protein, other trace nutrients and cellulosic roughage. Grass-eating in his Beagles was not observed extensively, but it did occur. What he did find, however, was the presence in the ova of the nematode, Capillaria plica, in the urine of nearly all his Beagles allowed access to grass. This parasite has not been recorded frequently in dogs, although well-known in both wild and captive foxes (Watkins & Harvey, 1942), and has the earthworm as its obligatory intermediate host.

The author has hitherto reviewed canine behaviour (Worden, 1959) and although the contribution was entitled 'Abnormal behaviour in the dog and cat' it did in fact summarize evidence to date on normal as well as aberrant behavioural patterns in both species and, to avoid repetition, is included as Appendix II to this thesis. Later behavioural studies that have special reference to the Beagle have been referred to in 3:6. The author finds it difficult to accept that the domestic dog is descended from other than the wolf, or a common wolf-dog ancestor, although the distinction may not be so important as Konrad Lorenz may have indicated, for jackals are not distantly related and reciprocal jackal-dog crosses have been reported. What is important in relation to the use of the dog as an experimental animal is the distinct contrast between recognized breeds in

their timidity or man-reaction. As discussed in 3:9, the Basenji, for example, has a much greater shyness at 5 weeks of age than the Cocker Spaniel, and this shyness is inherited on Mendelian lines, there probably being two genetic factors involved.

A knowledge of dog behaviour is an integral part of good dog management, and those who work daily with their charges, and who came to know them and to understand their reactions, preferences and rank-order, may detect early signs of experimental effect even before the scientist in charge.

McCay in 1943 produced a monograph on the nutrition of the dog that, in its later edition (McCay, 1949) remains a valuable reference source, while the nutritional requirements of the species have been summarized by Michaud & Elvehjem (1944), the National Research Council (1954), Bourne (1957), Schwick (1959) and Tagwerker (1965) and others. Practical dog feeding was discussed in detail by Whitney (1949) and by Abrams (1962, 1965). The present author has dealt with certain aspects of dog nutrition (Worden, 1960), with the feeding of laboratory dogs and cats (Worden, 1965) - see Appendices III and IV - and with the feeding of laboratory animals in general (Worden & Shillam, 1967), while Wolf, Della Rosa & Corbin (1970) have summarized relevant data with particular reference to the experimental Beagle, but the most comprehensive account of laboratory dog nutrition is that recently submitted in thesis form by Walker (1971).

Walker (1965) had earlier discussed the rearing of Beagles on controlled diets, including some observations on Miniature Poodles, and together with the author's colleagues he organized trials at HRC in which 4 pairs of Beagles received, in rotation, a purified or semi-synthetic reference diet in powder form (casein, sucrose, arachis oil, minerals, vitamins and solka-floc), a semi-solid diet (a 2:1 mixture

by weight of canned dog food and biscuit), and 2 complete dry diets in expanded pellet form, after which all animals received a nitrogen (N)-free diet. While the apparent digestibility of N was greatest on the reference diet, food consumption and bodyweight gain were lower than on the other diets. It was concluded that complete dry diets, the composition of one of which is given by Worden (1965) and by Walker (1971), are adequate for maintenance of laboratory dogs.

Some of the key questions are dealt with in Appendices III and IV, and need not therefore be repeated here. It may safely be said that, in practice, it is now possible to rely upon prepared 'complete' compressed diets for experiments lasting up to 2 years. The practice at HRC is to offer each dog 2 meals, each of 200 g. dry diet, a day, i.e. a weekly total of 2.8 kg. Mean food consumption in these circumstances has been summarized by Noel (1969) and is reproduced in Table XXXIV. It will be seen that the majority of males studied consumed all the food offered to them, while female dogs generally left small quantities and, that, once adult age was attained, the quantities remained constant. The mean food intakes for males and females combined show that at about 14 to 16 weeks of age, the weekly intake was 2.16 kg, with an increase to 2.7 kg when adult weight was reached. Noel points out that these quantities are not in accord with those recorded in the preface to Appraisal of Safety of Chemicals in Foods, Drugs and Cosmetics, the second

edition of which was published on behalf of the Editorial Committee of the Association of Food and Drug Officials of the United States, where the consumption of dry food by dogs weighing 10 kg was given as 250 g/day, or only 1.75 kg/week. This implies a change in the conversion from oral dosing on a mg/kg/day basis to dietary intake (in parts per million or p.p.m.) must, at least in the circumstances prevailing at HRC be changed, i.e.

(i) 1 mg/kg/day is approximately equivalent to 26 p.p.m. (and not 40 p.p.m.);

(ii) 1 p.p.m. is approximately equivalent to 0.04 mg/kg/day (and not 0.025 mg/kg/day).

The bodyweight changes of Beagles at HRC receiving this diet are shown in Table XXXV and in Fig. 5.

At HRC the author and his colleagues have studied the utilisation of various members of the vitamin B complex in several breeds of dogs (Worden, Waterhouse & Partington, 1952, 1954, 1955; Worden & Waterhouse, 1955; Worden, Waterhouse & Pulman, 1956; Heywood & Partington, 1971; Noel, Chesterman, Jolly, & Partington, 1971). Among the findings of interest are that the excretion of thiamine, aneurin or vitamin B₁ is not proportional to bodyweight but is dependent upon the activity of the dog and upon the volume of urine passed, there being considerably less fluctuation in urinary thiamine concentration than in urinary volume/day. Other workers had already shown that the composition of the diet affected the require-

ments for this vitamin: Arnold & Elvehjem (1939) found that when the fat content of the diet was increased through isocaloric replacement of the carbohydrate, the thiamine requirement could be lowered by some 66%. In studies at HRC designed to ascertain whether or not the recommended National Research Council (1962) requirement of 13.2 to 17.6 µg/kg bodyweight/day was adequate for the Beagle, groups were fed that in effect received amounts varying from 11 to 125 µg/kg/day. There was no clinical evidence of thiamine deficiency, but with the exception of the liver levels, which remained constant irrespective of thiamine intake, tissue levels (i.e. kidney, heart-muscle and skeletal muscle) were dose related. The levels were affected also by frequency of thiamine intake, being lower in those dogs dosed twice weekly than with an equivalent amount given in regular daily doses.

With vitamin B₂ or riboflavin, it was found that the urinary excretion could be altered by varying the environmental temperature. With an increase of approximately 20°F in the environmental temperature, the urine excreted/day was reduced to between one-third and one-half of the original volume. The concentration of riboflavin in the reduced volume of urine was, however, always more than twice that in the urine excreted at the lower environmental temperature.

In a group of Beagles maintained at HRC on a diet deficient in riboflavin, superficial corneal ulceration was recorded

in one animal, as already mentioned in 3:6. Of 7 dogs receiving diets containing sub-optimal amounts of riboflavin, 3 developed corneal opacities.

Worden et al. (1955) confirmed that small quantities of vitamin A are present in the urine of normal dogs, although the levels, which were found to vary from approximately 2.5 up to 50 I.U./100 ml, were of a lower order than that indicated by some earlier workers.

The nutrient requirements of dogs have been summarized by Walker (1971) and his conclusions are reproduced with due acknowledgement in Table XXXVI. Walker draws attention to errors that have occurred in the American literature on iron intake by the dog, due in part to an unusual formula for iron citrate that is neither the ferrous nor the ferric form and in part to apparent decimal errors in calculation.

The water requirements of the laboratory dog have been summarized by Worden (1960), which is reproduced as Appendix III to this thesis, and are commented upon also by Worden (1959) in his review of canine behaviour. These papers erroneously cite the name of Towbin (1955) as Tarbin: this author was responsible for showing that dogs consume larger volumes of water daily at higher environmental temperatures by taking more frequent drinks than by any change in the amounts of water taken at each drink, and that a given dog appears to favour a characteristic size of drink that is not markedly affected by total food or water intake. The

probable effect of kidney damage upon this pattern has been discussed by Worden (1959).

The nature of the diet is of importance in studies involving the long-term administration of test compounds. Thus Sullivan & Kane (1971) have reported that the toxicity of metalozone was more marked in Beagles receiving an all-meat diet than in those fed Purina Canine Checkers, diuresis and haemoconcentration appearing in the first week of medication. They conclude that the effects of metalozone were more severe in dogs receiving sub-optimal diets. There is the even more important factor of interaction between diet and test compound. Although specific evidence for the dog is not available in such clear-cut form, it is of note that in rats at HRC it has been established that the toxicity of a test substance may be either reduced or enhanced. Worden & Harper (1964) found that the respiratory stimulant, dimeflin, or 3-methyl-7-methoxy-8-dimethylamino-methylflavone hydrochloride, was much less toxic than when dosed orally, although it was established that it remained unchanged chemically in the diet. It seemed likely therefore that the absorption of the drug from the gastro-intestinal tract was retarded, thereby limiting its accumulation in the blood and in brain tissue. The converse was found to be the case with a phenothiazine derivative, dixyrazine, or 10-(2-methyl-3-1-[1-hydroxy-ethoxyethyl-4-piperazinyl] propyl) phenothiazine, employed therapeutically as a neuroleptic tranquillizer. Analysis of the test diet showed that only about 40% of

unchanged active ingredient remained so that in this instance the drug appeared to have undergone degeneration in the presence of organic food constituents and moisture, with the formation of more toxic degradation products.

TABLE XXXIV

Body weight changes (Beagle dogs)

Age (weeks)	Males			Females		
	No.	Mean weight (kg)	Gain per week	No.	Mean weight (kg)	Gain per week
14-16	139	5.76		139	5.33	
+ 4	139	7.18	354 g	139	6.58	312 g
+ 8	139	8.95	443 g	139	7.96	346 g
+ 12	139	10.40	363 g	139	9.00	260 g
+ 16	139	11.47	266 g	139	9.72	180 g
+ 24	139	12.50	130 g	139	10.51	99 g
+ 52	100	13.14	23 g	101	11.31	29 g

All dogs are obtained at 14-16 weeks of age

All dogs offered a complete dry diet (2800 g/dog/week) with milk supplements

All dogs were normal or 'controls'

TABLE XXXV

*Mean food consumption (Beagles) according to sexes
(in kilograms dry diet/dog/week)*

Duration of study* (months)	Mean intake and 95% confidence limits (CL)			
	Males		Females	
	Mean	(95% CL)	Mean	(95% CL)
1st	2.27	(1.6-2.8)	2.05	(1.4-2.7)
2nd	2.62	(2.1-2.8)	2.39	(1.8-2.8)
3rd	2.68	(2.3-2.8)	2.45	(1.9-2.8)
4th	2.73	(2.4-2.8)	2.55	(2.0-2.8)
6th	2.76	(2.7-2.8)	2.60	(2.2-2.8)
7-12	2.77	(2.6-2.8)	2.59	(2.1-2.8)

Note: Numbers = 130♂ and 130♀

Basic ration offered = 2800 g/dog/week (dry diet)

* Age on arrival = 14-16 weeks

TABLE XXXVI

Nutritional requirement

Nutrient intakes for dogs: summarised conclusions

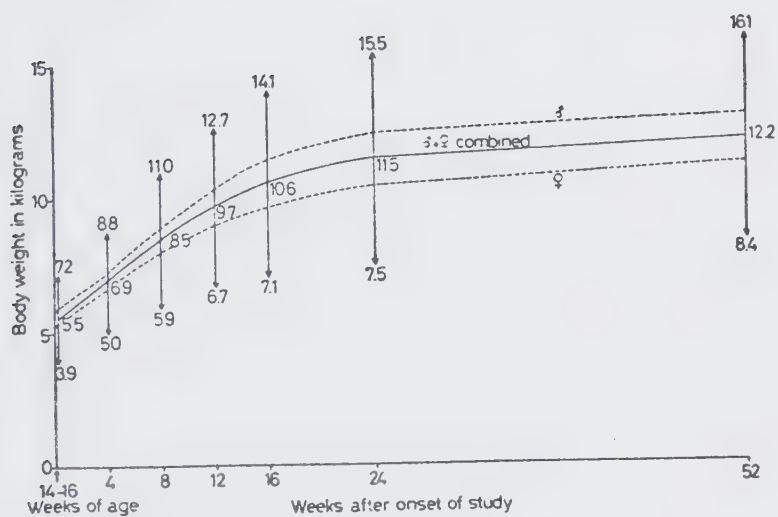
Nutrient	Amount for adult daily maintenance per kg. body wt.	Dietary level in dry rations (approx. 10% moisture)
Energy	See Table 1.2.A.	3500 kCal/kg
Protein (N x 6.25) ^a	5.0 g	20%
Fat	1.3 g	5%
Minerals ^b		
Calcium	250mg	1.0%
Phosphorus	200mg	0.8%
Magnesium	5mg	200 ppm
Sodium chloride	125mg	0.5%
Potassium	125mg	0.5%
Iron	3mg	120 ppm
Copper	200µg	8 ppm
Cobalt	50µg	2 ppm
Iodine	38µg	1.5 ppm
Fluorine	?	50 ppm
Vitamins		
Vitamin A	100 i.u.	4000 i.u./kg
Vitamin D	6.6 i.u.	264 i.u./kg
Vitamin E ^c	1mg	40 ppm
Vitamin K	5µg	0.2 ppm
Thiamin	25µg	1.0 ppm
Riboflavin	30µg	1.2 ppm
Nicotinic acid	250µg	10.0 ppm
Pantothenic acid	50µg	2.0 ppm
Pyridoxine	25µg	1.0 ppm
Choline	27.5µg	0.11%
Folic acid	4µg	0.16 ppm
Vitamin B ₁₂	0.5µg	0.02 ppm

^a - with N.P.U. not less than 50

^b - Manganese, zinc and other trace elements not included owing to lack of data

^c - as D - L - tocopherol

FIGURE 3



Mean body weights of Beagle dogs (with 95% confidence limits for combined males and females).

4 : 4 Administration and monitoring of the test
material

Worden, Noel & Jolly (1963) summarized the position in dogs as follows:

'Certain organ function tests require the administration of test substances, and these may be given either orally or by parenteral injection.

'Substances can be given orally in the diet, by stomach tube or by capsule. Dietary administration is the least time consuming also the least accurate, since the dose at any one time depends on the food consumption of the dog. This method therefore is not to be recommended and, since the dog is used experimentally in relatively small numbers, individual dosing is preferable. Passage of a stomach tube (usually a rubber catheter) is useful for giving fluids, emulsions or suspensions. Dangers however do exist; the catheter can pass into the trachea with consequent intrapulmonary injection, or the catheter may not reach the stomach and the oesophageal contents may be regurgitated and aspirated, with the same result. Administration by gelatine capsule is an easy procedure and appears to be without danger; however, it can only be used where the volume of substance is not too great.

'Parenteral injection may be intravenous, intramuscular, subcutaneous or intraperitoneal. The last appears to be quite suitable in dogs and almost equivalent to the intra-

muscular route as regards rate of absorption for water-soluble substances.'

The gelatine capsules employed at HRC are of size 000, which are administered without difficulty. The problems arise in the mechanical preparation or filling, which is under strict pharmaceutical supervision and which involves nearly 2 million capsules per annum.

In the case of intramuscular injections, it is the practice at HRC to limit the volume to 2 ml in order to lessen the risk of tissue damage due to the volume itself.

Noel has calculated the order of quantities of test compound required for the dog and other species. In the case of oral dosing he has based his computation on intakes of 60 mg/kg/day by a high level group, 30 mg/kg/day by an intermediate group and 10 mg/kg/day by a low-level group. A group comprising 3 male and 3 female Beagles or Corgis over a dosing period of 6 months would require a total amount of test compound of 0.9 kg, compared with 0.7 kg for a comparable experiment involving the same numbers of small baboons, 0.2 kg for a rat experiment with groups each of 15 males and 15 females, and 10.0 kg for a pig experiment involving 3 males and 3 females at each dose-level.

In the case of dietary administration, based on high, intermediate and low levels of 1,000, 100 and 10 p.p.m. respectively, the corresponding quantities for an experiment

of the same order of numbers would be 0.60 kg for the Beagle or Corgi, 0.45 kg for small baboons, 0.18 kg for rats and 2.0 kg for pigs. In a dietary experiment lasting 2 years these values would be increased to, respectively, 3.1 kg for the dog, 2.7 kg for the baboon, 1.9 kg for the rat and 18.6 kg for the pig.

The monitoring of the test substance is extremely important, but is a factor that is often overlooked. The failure of an animal to swallow a capsule is easily enough detected, provided that the administrator is vigilant, but where intubation or 'gavage' is employed great care must be taken to avoid passing the catheter or other tube involved into the trachea instead of the oesophagus. Many disasters and deaths in rodents and other species have resulted from this.

Chemical monitoring is equally important. Reference has been made elsewhere in this thesis to the findings of Worden & Harper (1963), who showed that breakdown may occur in the diet, sometimes with the formation of more toxic degradation products. Even when the test material is unchanged, its absorption from the food may be much lower than following intubation or dosing by capsule, hence the desirability of checking absorption by the determination of the test substance in the blood or other body fluids.

In long-term dietary studies with the dog at HRC it has been the practice, whenever suitable methods have been avail-

able, to examine the amounts in the diet after mixing and to follow blood levels. It is the author's opinion that terminal studies should include the examination of certain tissues for the presence of test material or its metabolites, to determine whether or not storage or accumulation has occurred.

The practice commonly adopted in the mixing of animal feedingstuffs of adding some colouring matter to a trace supplement, in order that the distribution of the latter may be visible, is not really applicable - or applicable in rare cases only, to long-term studies involving dogs. One problem in toxicological investigations is that the colouring matter - or other 'telltale' if other than visible identification is required - may have to be incorporated in the diet in amounts comparable with those of the test material itself. This means that a second material is being 'studied.'

4 : 5 Clinical observations, including ophthalmology
and electrocardiography

Worden, Noel & Jolly (1963, pp. 405-406) have already summarized the routine procedures to be adopted in the clinical examination of experimental dogs, as follows:

'When examining a dog it is best to have a routine which is strictly followed. The dog should be removed from its kennel and placed on a table under good light. The temperature is taken, and in a healthy dog this should be 38.3-38.8°C (101-102°F), although in puppies it may be as high as 39°C (102.5°F). At this time the consistency of the faeces can be noted. While waiting for the temperature to be recorded, the breathing of the dog should be observed and the heart beat counted. The respiration rate varies from eighteen a minute in small animals to twelve in large. The heart beat ranges from seventy to ninety a minute and is again faster in the small dog. The nervous or excitable dog will, however, show an increased heart beat and in hot weather a normal dog may pant at 100 respirations a minute. Panting, under other circumstances, may indicate respiratory distress or heart dysfunction.

'The head, nose, mouth and tonsils should then be examined. Older dogs develop deposits of tartar along the gingival border of the tooth; these deposits should be removed. The eyes should be clear and bright; a discharge from one eye usually indicates injury or the

presence of a foreign body in the affected organ. Discharge from both eyes is a sign of a systemic disturbance, possibly canine distemper or infectious hepatitis. The ears should be clean although a small amount of wax is normal. Dogs with flap ears, such as Spaniels, are very susceptible to ear infection since there is inadequate ventilation of the ear canal. The skin of the neck and back should be searched for parasites by removing the collar and passing the hand against the coat so that the skin can easily be seen.

'The hands should then be passed over the body of the dog and down each hind leg. Any lesions or swellings and joint distentions which are not readily apparent can be detected by touch. The hind feet and the pads should be carefully inspected; ringworm sometimes take the form of circular hairless patches at the junction of the claw and hair portion of the feet. The forelegs should be examined in the same fashion. The dog can then be clasped around the forelimbs and lifted upright to stand on its hind legs, so that the abdomen can be seen. In the male dog the sheath of the penis may show a slight soiling which is normal in some dogs, but any excessive discharge indicates an infection.

'With practice, this examination of the dog can be made rapidly but without missing any gross abnormality. Practice

will also make the minor abnormalities more 'conspicuous, and such factors as the condition of the coat, the looseness of the skin and the general reaction of the dog to the examination will aid in assessing the general health of the dog.'

At HRC the special construction of the outside exercising runs permits of individual and group observation. The latter is of significance when it may be practised, since it brings together animals that have been housed overnight and for much of the day in indoor pens, singly or in pairs. A variation in response of an individual to his fellows may be the first indication of some drug-induced change (or of a non-specific change or deficiency), and the research assistants who keep the dogs under constant supervision, and who of course know each animal intimately, are requested to note any apparent change in the behavioural pattern.

The eye has proved of outstanding importance during recent years, as has been emphasized by the writer's colleagues, Barnett & Noel (1969) and Dr. Ralph Heywood, who has specialized in both direct and indirect ophthalmoscopy (see Figs. 3 and 4). The following summary of the techniques adopted at HRC has been adapted from Heywood (1972), in preparation:

'The techniques used in veterinary ophthalmology are essentially those used for man. It is important to adopt a systemic procedure in the examination of the eye and to

ensure that the findings at each stage of the examination are recorded at the time of examination.

'The method of examination is based on simple optical principles, although sophisticated apparatus has been developed for use in the human field (Berens & Zuckerman, 1946; Duke-Elder, 1962). Animals may not be co-operative when making the examination and it is therefore important to keep the experimental technique relatively simple. Toxicological studies are made on groups of animals, therefore whatever techniques are adopted must be practical, not too time-consuming and applicable to the unanaesthetized animal.

'The general examination of the eye and its adnexa is usually possible with a minimum of restraint, under adequate diffuse day-light, or by using a simple pencil flashlight. Focal illumination with or without the aid of a loupe is not really applicable.

(1) Direct ophthalmoscopy

'The direct method is the one most frequently described as being most suitable for animals (Krawitz, 1965). By using the retinal reflex "opacities of the media" can be shown as shadows against the "red reflex": a simple black dot suggests a lens opacity. By correct use of the plus and minus lenses a series of examinations of the eye from fundus through the vitreous body, the lens, the chambers and finally

the cornea can be made. Direct ophthalmoscopy permits a very detailed examination of the eye, and assessment of the degree of myopia or hypermetropia.

(11) Indirect ophthalmoscopy

'The potential of this method of examination in the fields of veterinary ophthalmology was recognized by Rubin (1960) while clinical experience with the instrument has been described by Vierheller (1966). Indirect ophthalmoscopy is particularly advantageous in the examination of the eyes of animals on toxicological tests. The greater illumination provided allows one to examine the cornea, lens, vitreous and retina at greater speed than by the use of the direct ophthalmoscope. The modern binocular instrument allows stereoscopic vision, and this helps the recognition of lesions on the fundus and within the vitreous. Larger fields are visualised at one time, and the peripheral fundus can be examined with comparative ease. It is easy to compare the two eyes quickly.

'To make a critical examination of the eye, the inspection should be conducted in a darkened room. Mydriasis is necessary for observations on the lens and fundus field. With the indirect ophthalmoscope full dilation of the pupil is essential. Rubin & Wolfes (1962) tested eleven mydriatic

drugs in the dog and found tropicamide 1% to be the most useful. In the Beagle dog instillation of one drop of 1% tropicamide produces maximum dilation within twenty minutes, the mydriatic effect beginning to fade after 75 minutes..... In the dog, the rat and the monkey no side effects have been recorded following the use of tropicamide.

'To facilitate examination of the conjunctival epithelium and the cornea, fluorescein staining can be used, since damaged epithelium retains the dye. One drop of 1% fluorescein solution can be instilled into the conjunctival sac; to prevent infection it is better to use fluorescein strips applied to the conjunctiva, at the same time this technique establishes the patency of the tear duct.

'Slit lamp examination of the eye can be performed but not with sufficient ease to recommend its use in the routine ophthalmologic examination. Modification of the standard slit lamps and especially designed restraint have been recommended in animals (Uberreiter, 1959). Restraint of the animal is one of the main problems and anaesthesia or some degree of tranquillisation is considered necessary to make detailed examination.

'Martin (1969) has recently published his observation on the biomicroscopic examinations of twenty apparently normal dogs, and his findings revealed significant differences from those in the human eye.

'It is my practice to carry out slit lamp examinations to define more exactly lesions observed by the indirect ophthalmoscopic examination.

'Clinical records of each ophthalmoscopic examination should be kept, and it is useful to obtain pictorial evidence of clinical abnormalities and eye changes. The technique of photography should be kept as simple as possible.

Park et al. (1959) described a technique for photographing cataractous lens changes in animals. I have found the fixed focal beam camera (Raynor-Wray) to be the most useful piece of apparatus for photographing the eye and its adnexa, and with some experience very satisfactory photographs of the lens can be obtained.

'Lack of co-operation from the subject makes fundus photographs difficult to obtain. The first good fundic photographs in the dog were obtained by Catcott (1952) using the Nordensen retinal camera. Donovan & Wyman (1964) used the Noyori hand fundus camera and obtained excellent photographs; it was necessary however to use some methods of chemical restraint. The Kowa hand-held fundus camera was found by Barnett & Keeler (1967) to be the most satisfactory for all animal species. The Kowa 2 camera has been found to produce very satisfactory results in the dog.

'A camera has been incorporated into the optical system of the Gambs slit lamp which allows one to take photographs during the course of the examination. There are diffi-

culties in obtaining satisfactory results due to the fact that the time exposures are usually required of up to one or two seconds, and this is not practical on the unanaesthetized animal.

'The intraocular pressure can be assessed by an indirect method, the tonometer is an instrument devised for the measurement of intraocular pressure from determination of corneal tension. The Schiötz tonometer is the usual model used in veterinary ophthalmology, though experience with this instrument is very limited (Magrane, 1951; Bryan, 1965).

'This indirect technique is applicable to the dog and to the monkey. The recording is best made using only topical anaesthesia, and for this purpose propacaine hydrochloride 0.5% is the best anaesthetic. With the dog the head is held so that the eyes are directed upwards and the tonometer is allowed to rest by its own weight upon the cornea. Ideally the instrument should rest on the centre of the cornea, but in practice the upper third of the cornea is the site used.'

Dr. Heywood's published data on developmental changes in the lens of the young Beagle dog (Heywood, 1971) and his joint publication on ocular lesions induced by vitamin B₆ deficiency in the Beagle (Heywood & Partington, 1971) have already been referred to in 3:6.

At HRC, Osborne (1972) is preparing a thesis on electrocardiography of the dog and other species, and is adding to the information summarized by Noel (1969) and by Ettinger & Suter (1970), and to his own preliminary findings (Osborne & Leach, 1971). A typical normal electrocardiogram of a male 2-years-old Beagle is given in Fig. 6, and one taken on the same animal directly following the intravenous administration of 4 units of vasopressin is given in Fig. 7, showing the resultant bradycardia, the peaked T waves indicative of anoxia and the arrhythmia in lead II. The author is indebted to Mr. Osborne for these illustrations, and for his valuable contributions towards a better understanding of electrocardiograms in this species.

Osborne (1970) has designed a restraining apparatus which can be utilized by the cardiograph operator alone. 'It is a comfortable device which maintains the animal in an almost constant position without causing undue stress, is easily assembled, and can be situated close to the cardiograph'. Essentially it consists of a U-shaped wooden console, approximately 44 cm x 46.5 cm x 88 cm high, having a base line with a 10 cm layer of synthetic foam rubber. Two casters at the base of the rear of the console and a handle at the top render the apparatus portable. The dog is lifted backwards into the console and allowed to sit. Its forelimbs are then lifted, using the left arm, until the animal is almost vertical. With the right arm the appropriate wooden restraining shelf is selected and slid into position just below the

axillae. The animal is then allowed to rest with its forelimbs on top of the shelf, which is locked in position with a double wooden peg. If further restraint is required another shelf can be fitted and similarly fixed above the animal's thighs, but less than 2% of all recordings have necessitated this. The shelf or shelves are positioned so that their padded inner rims fit snugly around the chest and thighs. 'So positioned, the animal can make little vertical, lateral, backward or forward movement.' Nearly all Beagles find this 'begging' position produced by this apparatus quite comfortable. 'Using the apparatus described, satisfactory ECG's can be recorded with minimal stress of the animals as judged by the consistency of traces obtained and the tolerance with which the animals submit to repeated measurements. Of 800 recordings obtained so far, no evidence has yet been found of artifacts induced by either the method or apparatus'.

The neurological examination of the experimental dog was summarized by Worden, Noel & Jolly (1963) as follows:

'Canine neurology and such specialized techniques as electroencephalography in the dog are still in their early stages. Radiological techniques involving the use of radio-opaque materials and air have been described by Magrath (1956).

'It is frequently desirable to know the drug level in the cerebrospinal fluid in order to demonstrate passage of the blood-brain barrier. The lumbar route is not possible

in the dog, so that cisternal puncture is necessary. Following the method described by Cobb (1960), the animal is seated on a chair at a small table in a begging position. The forelimbs are fixed to the opposite legs of the table and an attendant holds the head horizontal. If the dog is in the correct position the operation is simple to perform. Using a 1 in (2.5 cm) wide-bore needle, the point of the bevel is filed away producing a shallow V-shaped bevel. The needle with its stylette is inserted in the midline at a point just above the level of a line joining the upper border of the wings of the atlas, which is easily felt. Penetration of the atlanto-occipital membrane is felt, but not entrance into the cisternal space. The needle should not go more than $\frac{1}{8}$ in (3 mm) past the membrane. When the stylette is removed, cerebro-spinal fluid should flow. Rotation of the needle and abdominal pressure are two useful aids to increase the flow, which once established can be aided by very gentle suction from a syringe.'

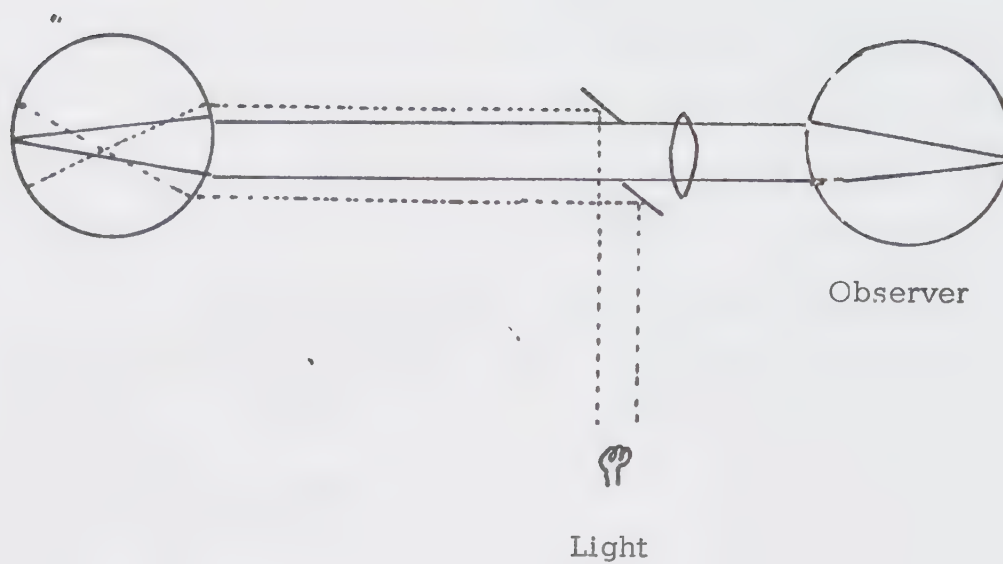
Since that review was written there has appeared the textbook edited by Hoerlein (1965), the second edition of which has been published in 1971. The neurological examinations described therein are perhaps more adapted to veterinary clinical practice, but there is a valuable chapter by Redding (1971) on the anatomy and physiology of the canine nervous system, as well as chapters by the same author on the examination of the living animal (Redding, 1971b) and on electroencephalography (Redding, 1971c), and a section by Rubin (1971) on neurophthalmology.

FIGURE 6:

ELECTROCARDIOGRAM OF 2-YEARS-OLD BEAGLE,
BEFORE TREATMENT WITH VASOPRESSIN

Direct Ophthalmoscopy

Fig. 4



Indirect Ophthalmoscopy

Fig. 5

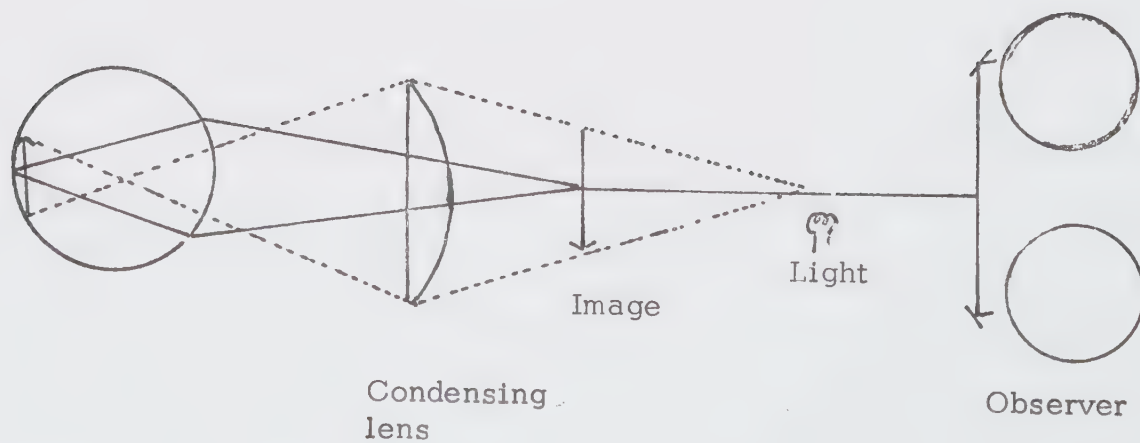
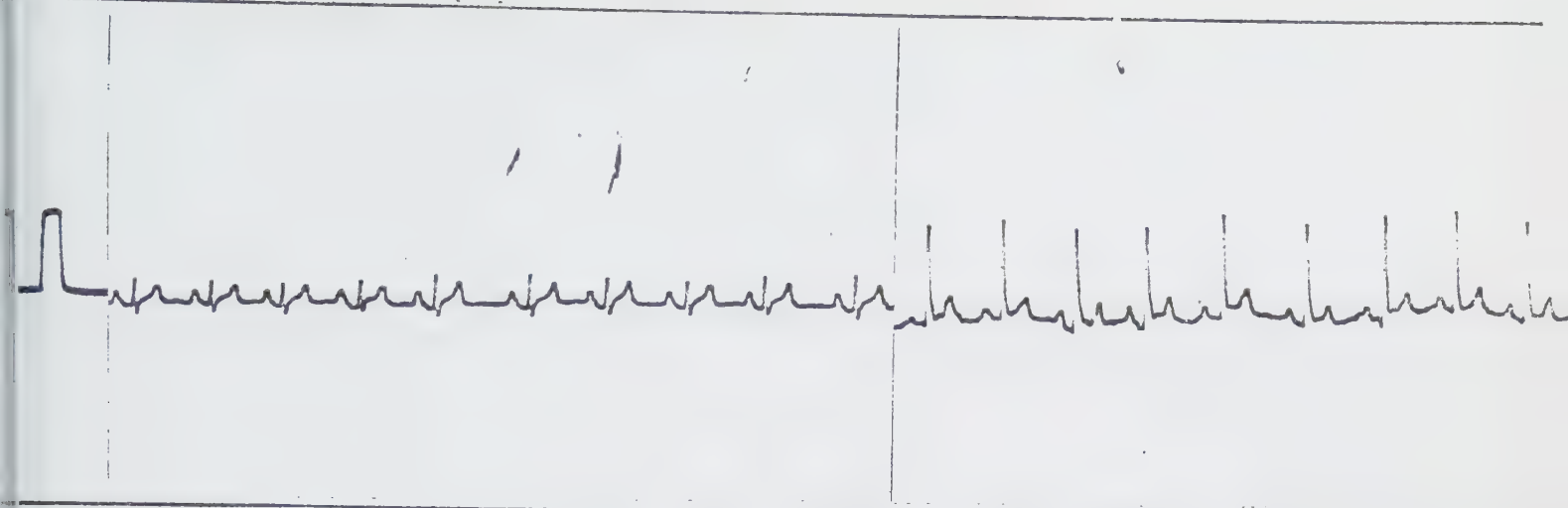
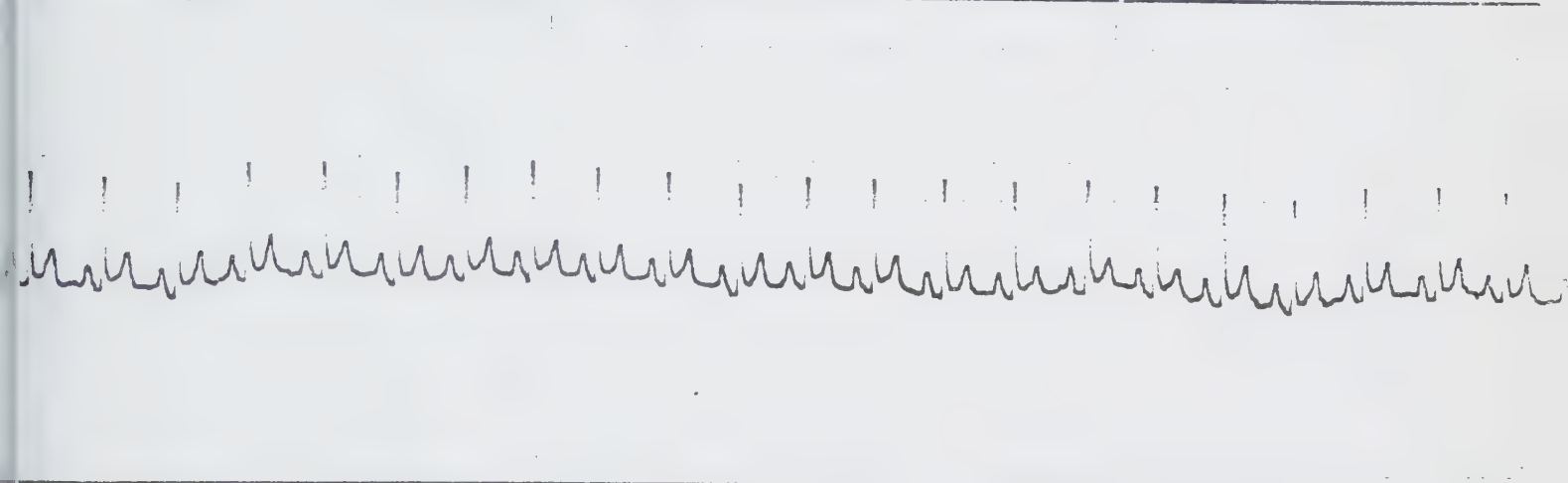


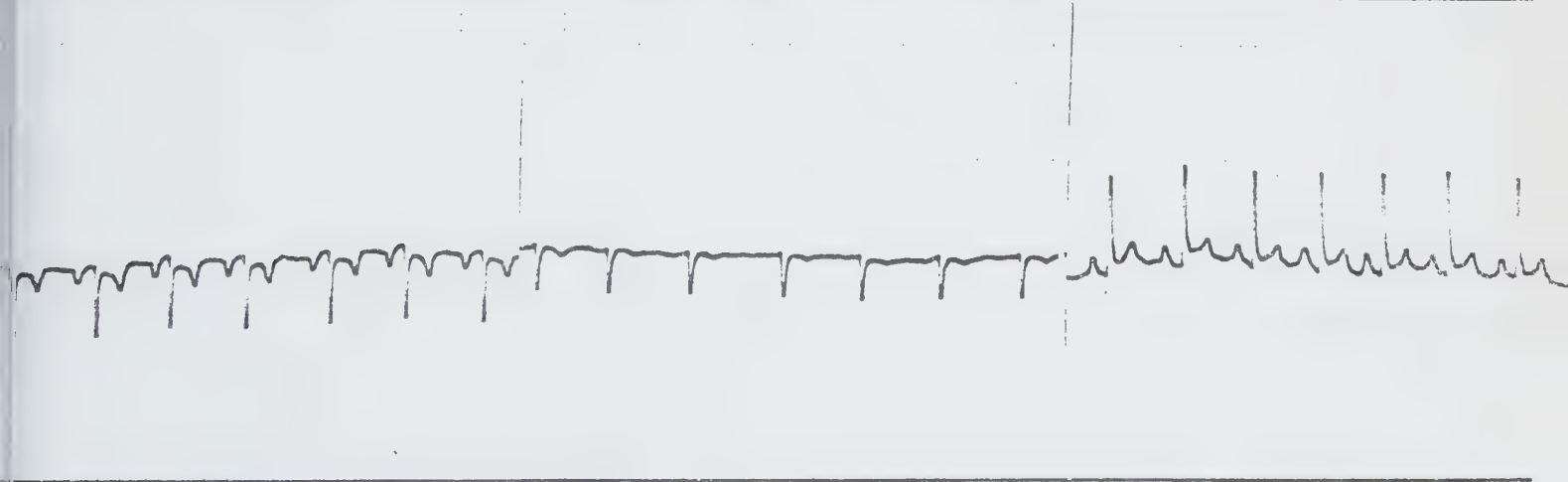
Figure 6



Standardization LEAD 1 LEAD 3



LEAD 2



aVr aVl aVf

R-R Interval	9.2 mm	.37 secs	Heart Rate	163	per minute
P-R Interval	.10 secs	Q-T Interval	.16 secs	QTc	.26

FIGURE 7:

ELECTROCARDIOGRAM OF BEAGLE (AS IN FIG. 6)
DIRECTLY AFTER INTRAVENOUS ADMINISTRATION OF
4 UNITS OF VASOPRESSIN, SHOWING BRADYCARDIA,
PEAKED T WAVES INDICATIVE OF ANOXIA AND
ARRHYTHMIA IN LEAD II

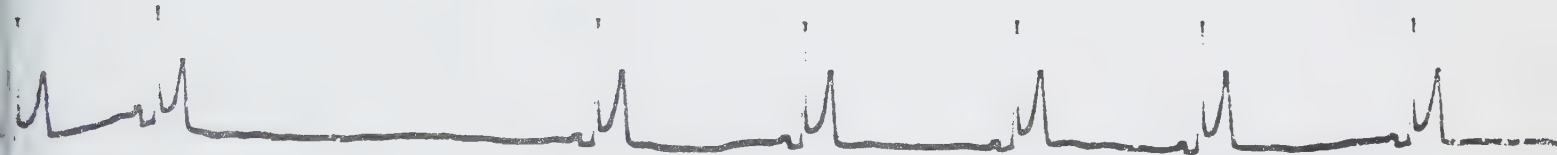
Figure 7



Standardization

LEAD 1

LEAD 3



LEAD 2



aVr

aVl

aVf

R-R Interval	27	mm	1.08	secs	Heart Rate	56	per minute
P-R Interval	.10	secs	Q-T Interval	.20	secs	QTc	.19

The taking, examination and interpretation of blood samples from the species employed in long-term toxicity studies - with notes on procedures involved in nutritional investigations - have already been summarized by the present author (Worden, 1970, pp. 107-141) and by his colleague Street (1970). The investigations dealt with, including the expression or units employed, include haematocrit or packed cell volume, PCV (% erythrocytes); haemoglobin, Hb (g/100 ml); methaemoglobin, MetHb (g/100 ml, or % Hb); erythrocyte sedimentation rate, ESR (mm/hr); erythrocyte count, RBC (10^6 /cmm); total white cells, WBC (10^3 /cmm); differential WBC counts (10^3 /cmm or % of total WBC); reticulocyte count (% RBC); platelet count (10^3 /cmm); platelet aggregation; platelet electrophoresis (μ sec/V/cm); prothrombin time (seconds); clot formation and retraction; plasma urea (mg/100 ml); plasma sugar as total reducing substance (mg/100 ml); plasma sugar as true glucose (mg/100 ml); serum total protein (g/100 ml); serum protein fractions (g/100 ml); serum cholesterol (mg/100 ml); serum bilirubin (mg/100 ml); serum alkaline phosphatase, SAP (King Armstrong or KA units/100 ml); serum glutamic pyruvic transaminase, SGPT (Sigma Frankel or SF units/100 ml); serum glutamic oxaloacetic transaminase, SGOT (Sigma Frankel or SF units/100 ml); serum isocitric dehydrogenase, ICD

(Bell & Barron units); bromsulphthalein or BSP clearance (% retention of dose); phenol red excretion (% retention of dose); serum sodium, potassium, chloride and bicarbonate (mEq/litre); serum calcium, magnesium, inorganic phosphate and iron (mg/100 ml); plasma protein bound iodine (μ g/100 ml); plasma cholinesterase (μ mols/ml/minute); erythrocyte cholinesterase (μ mols/ml/minute); serum ornithine carbamyl transferase (μ mols ammonia/minute); serum lactic dehydrogenase (International units/100 ml); blood pyruvic acid (mg/100 ml); serum amylase (Somogyi units/100 ml); serum formol-stable acid phosphatase (King Armstrong or KA units/100 ml); serum total lipids (mg/100 ml); serum triglycerides (mg/100 ml); serum total fatty acids (mg/100 ml); and serum phospholipids (mg/100 ml).

Some normal ranges of values for experimental dogs have already been tabulated in Chapter 3, and it remains only to make specific comments upon the dog, especially in those instances in which the normal range or the interpretation may differ from man or from other species of experimental animal.

Since Beagles may have an inherited Factor VII deficiency, all incoming dogs at HRC are screened for this defect, using the one-stage prothrombin test, combined with whole blood clotting time or partial thromboplastin time when the configuration or characteristics of the test compound would appear to indicate such a need.

The dog, in common with most laboratory species, has blood-clotting times much quicker than those of man, and it is the standard practice at HRC to employ an automatic fibrometer, thereby obviating between-observer differences.

The elucidation of the factor or factors responsible for a clotting defect is undertaken by the addition to the reaction mixture of samples known to be deficient in the specific factor, e.g. Factor VII from deficient dogs or Factor V, depleted by storage.

ESR in the healthy dog has usually almost a nil normal value.

There have been considerable difficulties to date in adapting Coulter counters to platelet counts in dogs and several other laboratory species, for which phase contrast microscopy remains the standard method, although Mr. Alan E. Street at HRC is obtaining some encouraging results by a method that avoids the brief centrifuging that is usually employed to reduce the numbers of RBC's.

The prothrombin time of the dog is usually of the order of 7.2 seconds, or about half the 12 to 14.5 seconds accepted as the normal range in human medicine.

Blood urea concentration appears to be of less value in the dog than in man as an indicator of renal function, especially when minimal changes are being investigated.

Street, Chesterman, Smith & Quinton (1968) showed that, in two colonies of Beagles, blood urea levels were raised significantly after feeding, and might even double over a 6-hour period, returning to pre-feeding levels only after some 16 hours. This postprandial rise has been noted by other workers and Vogin, Skeggs, Bokelman & Mattis (1967) even proposed a liver function test for the Beagle, based upon the elevation of serum urea following administration of a test meal, reporting that the elevation was reduced significantly by carbon tetrachloride or by ethionine. In their clinical study of canine nephritis, Richards & Hoe (1967) found that initial blood urea values were an unreliable index of prognosis, although serial estimations were satisfactory.

Climatic factors may also influence blood urea concentration in the dog. 'On the second day of a short warm spell at Huntingdon during the early days of July, 1968, when the shade temperature was well over 90°F, blood samples taken from some 30 Beagles had values averaging 39% higher than those taken two days later, by which time the shade temperature was below 75°F'. (Worden, 1970). Street (1969) considers that, owing to the high reserve capacity of the kidney, blood urea level in the dog is not elevated significantly until renal damage is well advanced. Harvey (1969), however, has records of dogs with relatively high plasma urea levels, which, at post-mortem examination, showed evidence of relatively mild renal damage.

In the dog, as in the baboon, the fasting level of non-glucose reducing substance (as determined by difference) is of the order of 7 to 8 mg/100 ml.

The Millipore Phoroslides system outlined in Bulletin PE-1 (Millipore Corporation, Bedford, Massachusetts, U.S.A., 1967) has given good separation of the major protein components of dog serum after only about 16 minutes exposure to 100 v. With this procedure, however, the dog has a higher recorded α_2 globulin fraction but a lower β -globulin fraction than with paper electrophoresis.

The dog, like the rat, has a low renal threshold for bilirubin. Hoe & Harvey (1961) found that while serum bilirubin levels were raised in many cases of liver dysfunction in the dog, they were often too near to, or even within a normal range of 0 to 0.6 mg/100 ml to be of real significance, and that values greater than 1.0 mg/100 ml were rare. Harvey (1967) found that it was not uncommon for dogs that were clinically healthy and apparently biochemically normal to be excreting sufficient bilirubin in the urine to give a ++ reaction to Fouchet's reagent. Experience at HRC has amply confirmed these findings, the only discrepancy being that the 'normal range' of serum bilirubin in the dog would be regarded as from 0 to 0.3, rather than 0.6, mg/100 ml. In several chronic toxicity studies in the dog at HRC, in which there has been evidence

of a dose-related but low degree of anaemia, with serum enzyme measurements within normal limits, serum bilirubin has been found to be slightly raised, due presumably to haemolysis.

For the estimation of alkaline phosphatase, dog serum is diluted in the ratio of 1:5. 'Although the blank values of these diluted samples have never been found to exceed 1 to 1.5 KA units/100 ml, their continued use is regarded as justified, especially as many drugs or their metabolites are phenolic in nature and could cause falsely high values.... The normal range of values found in dogs employed for chronic toxicity studies, which are at least 16 weeks of age before administration of the test compound is commenced, is akin to that of human patients' (Worden, 1970). Harvey (1967) has given a graphic illustration of the way in which the values for younger puppies have a wide scatter but tend to be several times the adult level, some values being over 40 KA units/100 ml, with a rapid decrease to normal adult levels of 8 KA units or below by about 12 weeks of age (See also the data in Table XXIV). SAP is not organ specific and, as Harvey has pointed out, it is not found in appreciable amounts in the liver. High levels in the dog may be associated with diseases other than those directly affecting the liver, e.g. those of the skeletal system.

Serum glutamic pyruvic transaminase is, however, virtually liver specific in the dog and in man (Cornelius,

Bishop, Switzer & Rhode, 1959). Serum glutamic oxaloacetic transaminase is not liver specific, but is frequently raised in liver disease. Its main application in human medicine has been related to the diagnosis of myocardial infarction, and in the dog it appears to have yielded useful information in a number of toxicity studies.

Serum isocitric dehydrogenase is regarded in human medicine as a useful indicator of active hepatocellular damage, and at HRC it is preferred to BSP clearance as supporting evidence for liver damage in the dog as indicated by other investigations. The normal values change with age: a young dog has usually an ICD of from 20 to 30 Bell & Barron units/100 ml, whereas by 12 to 18 months of age the level has fallen to about 10 units/100 ml.

BSP clearance is of value in veterinary diagnostic medicine, and Harvey (1967) has described its application to the dog. In experimental studies, however, its usefulness is limited by its extremely rapid excretion, and in the dog - and other laboratory animals - its clearance takes place so quickly that, even with relatively increased amounts of the dye, a circulating level of 100% is virtually unobtainable. Klaassen & Plaa (1967) studied the liver storage of BSP, its metabolic conjugation with reduced glutathione, and its excretion into the bile, either conjugated or unchanged, in the dog, rat and rabbit, and found that each species excreted less than 5% of the test dose in the urine. Liver storage

was about 5 times as great in the dog as in the rat or rabbit, but the capacity of the dog liver to conjugate BSP was less than that of rabbit liver and only about $1/80$ of that of rat liver. About 70% of the BSP appearing in the bile was metabolized in all 3 species, but the rate of appearance of BSP in bile and the bile-flow rate itself appeared to be the most important factors governing the removal of BSP from the bloodstream.

At HRC the dose of BSP employed for the dog is 20 mg/kg bodyweight, compared with the usual rate of 5 mg/kg in human patients.

The difficulties encountered with BSP are even greater with indocyanine green, although Vogin, Scott & Mattis (1965) and Vogin, Skeggs, Bokelman & Mattis have investigated clearance of this dye as an index of hepatic function in the Beagle.

There is an overall trend towards a metabolic acidosis in nephritic dogs (Richards & Hoe, 1967), and the more widespread nature of possible disturbances during toxicity experiments renders it desirable to examine serum sodium, potassium, chloride and bicarbonate in this species.

Serum ornithine carbamyl transferase and serum lactic dehydrogenase have been employed at HRC for certain drug toxicity studies in the dog, but are not routinely incorporated

in the majority of protocols. Circulating cholinesterase levels are followed when organophosphates or carbamates are under investigation, and may be considered also as possible indicators of liver damage, during which they tend to become depressed.

At HRC attempts have been made with the aid of serum formol-stable acid phosphatase determinations to investigate the significance of prostatic enlargement in the dog that appears to be dose dependent, with a view to excluding carcinomatous change.

Normal serum amylase values in the dog are about twice those in man but much lower than in the rat, typical values being of the order of 300, 150 and 2,500 Somogyi units/100 ml respectively.

The normal circulating levels of total lipids appear to be of much the same order in the dog as in man, whereas in the baboon they tend to be somewhat lower.

Data on haematological and blood chemical values obtained in dogs at HRC, or with Beagles included in Andersen's (1970) book, are incorporated in Tables III, IV, VI, VII, VIII, IX, XV, XVI, XVII, XVIII, XIX, XX, XXI, XXII, XXIII, XXIV and XXV.

The general principles relating to the collection and examination of urine and faecal specimens have been reviewed by the present author (Worden, 1970), while the practical issues involved in the case of nutritional studies involving the dog have been discussed by Worden (1939), Worden, Waterhouse & Partington (1952, 1954, 1955), Worden, Bunyan, Davies & Waterhouse (1955), Worden & Waterhouse (1955, 1956), Worden, Waterhouse & Pulman (1956), and Taylor, Worden & Waterhouse (1958, 1959). The usual method for the collection of specimens in toxicity studies was noted by Worden, Noel & Jolly (1963), but since that time the author's colleague, Dr. P.R.B. Noel, has devised a much more satisfactory means of converting normal pens into metabolism units, thereby obviating many difficulties (see Worden, 1970).

Single specimens of urine that are not easily obtained otherwise may be withdrawn by catheterization, and Murlin, Hayes & Johnson (1953) employed this procedure for their studies on the biological value of protein and its correlation with the percentage of urinary creatinine. Samples in the female are most easily obtained with the aid of a Kushev's illuminated speculum as described by Singleton (1959), for which anaesthetization is unnecessary and the only practical point to remember being that the urethral orifice lies high up the anterior vaginal wall, some 2.5 to 4 cm from the

introitus, i.e. about halfway up the length of the vagina.

Worden (1939) drew attention to the difficulty of obtaining adequate 24-hour specimens of urine in metabolism cages to which a dog had been introduced. Worden, Noel & Jolly (1963) recorded successful single sample collection in Gorgis with metabolism cages measuring 76 x 76 x 108 cm, but Worden & Waterhouse found that while cages measuring 108 x 108 x 92 cm were adequate for total urine collection over periods of from 5 to 12 days in dogs weighing up to about 20 kg, they were unsuitable for larger dogs, for which they described a really large cage, with a floor space of approximately 200 x 140 cm and an internal height of over 120 cm, and with several special features to overcome problems of contamination of the urine and faecal specimens, to permit of the easy weighing of the dog within the cage, to permit the dog to rest and to help to control the environmental temperature within the cage, for in some of their earlier studies they had recorded that an increase in environmental temperature of the order of 20°F decreased the 24-hour urine output to between one-half and one-third of its earlier volume. They had found also that the daily urinary excretion of riboflavin was about 75% greater at the 20°F higher environmental temperature.

Kleitman (1927) reported great variation in the quantity of water consumed by individual laboratory dogs from day to day. His dogs were not normally fed on Sundays

and from his paper it may be calculated that dogs weighing from 8 to 14 kg, within which range individual consumption did not appear to be related to bodyweight, the mean water intake on weekdays averaged 352 ml, that on Sundays only 64 ml and that during periods of fast 148 ml. He believed that the marked decrease in the water intake in starvation was probably one of the causes of a gradual deterioration of the salivary condition reflex. Tarbin (1955) showed that dogs respond to higher environmental temperatures by taking more frequent drinks rather than by any change in the amount of water taken at each drink. Indeed a given dog appears to favour a characteristic size of drink that is not markedly affected by total food or water intake. Adult dogs with kidney damage may, however, take very long single drinks (Worden, 1959).

False results, particularly when urinalysis is being used to help to investigate renal or hepatic function, may arise from the use of detergents in cage washing, from a failure to employ suitable containers (e.g. disposable wax cartons), or contamination with faeces, food or blood (e.g. from a female in oestrus).

Dog urine is normally acid in reaction, with a pH of about 6.0. The colour and volume of every sample should be noted and its reaction determined with the aid of a pH meter. The specific gravity may be determined hydrometrically or, if smaller specimens than 25 ml are available,

by weighing in a pycnometer. When a tiny volume only is available, the determination may be made with a refractometer.

Richards & Hoe (1967) have shown the relationship that exists between the initial urinary specific gravity values and the degree of uraemia in their long-term study of renal disease in the dog.

Routine screening tests for reducing substances, true glucose, ketone bodies, bile salts, bile pigment and free haemoglobin in urine may be carried out with reagents that are available commercially (e.g. those supplied by Ames) but any positive results of potential significance should be cross-checked. Many dogs that appear to be clinically normal excrete sufficient bile pigment to give a ~~++~~ positive reaction.

A common screening procedure for urinary protein is the Albustix technique, but for quantitative purposes it should be precipitated with salicylic acid (Worden, 1938) and the resultant turbidity compared with protein standards. Typing of the protein by electrophoresis usually requires prior concentration of the urine sample. A rapid and satisfactory concentration is achieved at HRC by a simple dialysis, with the insertion of a small amount of carbowax into a membrane sac which is then placed within a conical centrifuge tube containing the urine sample.

In toxicity studies, the examination of the deposit obtained on centrifuging a urine sample is a procedure that requires much experience and is full of difficulties. Light centrifuging for 10 minutes at 1,000 r.p.m. is all that is required, and after decanting the supernatant fluid it is helpful to stain the deposit with methylene blue or to employ phase contrast microscopy in order to differentiate the cell types. Unusual crystalline forms often represent the compound that has been administered.

'The most useful procedure yet employed for investigating possible renal damage is the urinary concentration test. After determination of the urinary specific gravity, water is withheld from the animal for a period (usually overnight) of about 16 hours, and the urinary specific gravity again measured as an index of renal concentration. In the dog, the value after the period of deprivation should be of the order of 1.040 or above. Values of between 1.035 and 1.040 are regarded with suspicion, and consistent values of below 1.035 may be regarded as indicative of renal damage or impairment of function (Worden, 1970, p.103). Balazs, Sekella & Pauls (1971) have reached similar conclusions in their studies on Beagles, in which values of above 1.039 were regarded as implying a normal renal concentration ability. The mean of the highest values they recorded was 1.058 $\pm$ 0.008.

The determination of urinary glutamic ~~oxalic~~acetic trans-aminase, together with a quantitative cell count as described by Balazs, Hatch, Zawidzka & Grice (1963) for the rat, has been suggested as a means of assessing marginal cases of renal damage, but at HRC it has not been regarded as worthy of replacing simple water deprivation and specific gravity determination. However, the author's colleague, Mr. Alan E. Street, is reinvestigating the possible value of this determination in the dog.

Urinary creatinine determination is regarded as of value as an index of the completeness of a 24-hour collection when, e.g. electrolyte excretion is being recorded, but its relative insensitivity does not justify its use as a means of detecting renal damage. The technique for plasma creatinine, adapted to the Technicon Auto-analyser, works well on urine with only minor modifications.

Ewald (1967) obtained renal function values from studies on 6 unanaesthetized littermate female Beagles when they were between $9\frac{1}{2}$ and $17\frac{1}{2}$ months of age, with the following mean values, all in terms of ml/minute/square meter of body surface area: glomerular filtration rate, 94.6; effective renal plasma flow, 251.82; effective renal blood flow, 397.74; osmolar clearance 2.48; free water clearance, 7.67. He recorded also a mean value of 38.0 for the filtrate fraction and one of 13.02 mg/minute/square

meter of body surface area for the tubular maximum of para-amino hippurate. His studies illustrate, however, the great difficulties that would be encountered if any attempt were made to apply these measurements routinely in long-term studies, and he himself states that: 'The greatest components of variability within the experiment were the observations obtained on different days on the same dog.'

Worden, Bunyan, Davies & Waterhouse (1955) confirmed that normal dogs excrete small quantities of vitamin A in their urine, the values they obtained ranging from 2.5 to 47.5 I.U./100 ml, or 14.3 to 235.0 I.U./day. Earlier workers, including Lawrie, Moore & Rajagopal (1941) had reported higher quantities, although failing to detect urinary excretion of the vitamin in normal human beings, and Worden et al. suggested that these higher values might have been associated with chronic renal disease.

Faecal samples are obtained fairly easily with the metabolism cages already referred to, and may be employed for nutritional studies provided that additional care is taken to prevent contamination with food, urine or blood.

In toxicological studies, it is a frequent procedure to look for the presence of occult blood, but with dogs - as with rats - faecal homogenates almost invariably give positive reactions to the standard techniques. At HRC it has been confirmed that the sensitivity of the ortho-

toluidine method of Smith (1958) may be reduced satisfactorily for routine use with the dog and most other laboratory species. 'Normal faecal homogenates from each species are boiled and filtered and the resultant filtrate is titrated against reagent concentration until the reaction after exposure for 2 minutes is deemed to be just negative. This concentration serves as a standard for test samples, and all reactions taking longer than 2 minutes to develop are regarded as negative' (Worden, 1970, p.106).

Mr. Street has applied the method of MacLagan (1946) for the estimation of faecal stercobilinogen as an index of suspected liver damage to both dog and rat specimens without apparent complications. Terwens alkaline phenolphthalein has been found to be a satisfactory standard, and colour matching by eye to be adequate.

4 : 8 Pathological procedures and interpretation,
including post-mortem examination, organ weight
analysis, histology, histochemistry and sub-
cellular investigations

'It is an essential part of a long-term toxicity experiment that any animal dying (or sacrificed when moribund or suffering) during the course of the investigation, and all of those surviving to the prescribed limit, should be subjected to a detailed post-mortem examination' (Worden, 1970, p.142). 'The practice of eviscerating animals and preserving the contents of the three body cavities in acid formaldehyde for later sampling of tissues is an unacceptable procedure. The mistakes made at autopsy can never be corrected and the successful completion of a toxicity experiment depends on proper dissection and fixation technique' (Benitz, 1969).

The numbers of animals involved may render it difficult to conduct a series of post-mortem examinations to the standard indicated by Innes (1930), but it is desirable to work to a schedule and to inspect certain key areas closely, including the abdominal, thoracic and cranial cavities. 'The most careful microscopic investigation cannot substitute for a thorough gross autopsy. For instance, in a severely **anaemic** animal the gross dissection of the gastro-intestinal tract may reveal bleeding ulcers. If this point had been

missed during the gross examination the most elaborate microscopy of hemopoietic tissue would be of little, if any, value. Other examples of this kind are the condition of the common bile duct, the portal vein and the ureters. It is also easier to estimate the quantity of lymphatic tissue in an animal during the autopsy rather than in histological sections' (Benitz, 1970).

At HRC, the actual post-mortem examinations are conducted largely by research officers and research assistants, working under the supervision of medical and veterinary pathologists, who are available to record comments (on tape recorders) of any macroscopic abnormalities or other features of apparent significance or interest. The various organs are then, when necessary, dissected free from fat, weighed, and preserved for histological examination. Specimens for histochemical or other form of subcellular study are taken and dealt with as soon as practicable. In the post-mortem rooms there are available series of pre-printed cards or labels, each bearing the name of the organ or tissue to be preserved, and this enables the organs and tissues to be identified without doubt and, at the same time, to check that every required specimen has been taken from each dog.

Some authors have challenged the value of routine histopathological study, and even Barnes & Denz (1954) raised certain doubts, emphasizing the differences in the approach

by different pathologists to the problems of interpretation and the difficulties of distinguishing between naturally-occurring disease or the effects of ageing from the effects of the test material. They suggested that, at the time when their review was compiled, the list of important histological findings in chronic toxicity tests would have been a short one and would have included liver tumours induced by selenium, thiourea, dulcin and butter yellow, renal damage after ethylene glycol and sulphonamides, liver lesions at the higher levels of DDT and BHC, and adrenal necrosis in the dog after DDD.

The problem of interpreting histological changes is exemplified by the report of Dallemagne, Gerebtzoff & Philip~~gott~~ (1950), who described fat deposits in the kidneys of dogs to which hexachlorocyclohexane or BHC had been fed. Silver (1951) stated that fat was normally present in the renal tubules of the dog and suggested that the illustrations of Dallemagne et al. did not appear to show any more fat than would normally be encountered. It is difficult to escape the conclusion that the report of Dallemagne et al. did not involve adequate use of control material.

Those concerned with experimental pathology and its interpretation have not all agreed upon the extent to which histological examination should be adopted. Frazer (1958) felt that it could be limited to the liver and kidney, while Boyd (1968) suggested limitation to grossly abnormal

organs, supplemented by material from two or three representative animals. Abrams, Zbinden & Bagdon (1965) listed 18 tissues they felt should be examined routinely, while Benitz (1970) advocated the preservation of as many tissues as possible, and lists an extensive incidence of drug-induced pathological changes encountered in his own laboratory. Although ~~extensive~~ histology may add some months to the reporting time, it is considered a necessary procedure at HRC, and this view appears to be shared by such bodies as the United States Food and Drug Administration and by the Committee on Safety of Medicines in the U.K. At HRC also the view is taken that detailed histopathological study is often essential to defining the nature of the actual lesion, as was clearly indicated in the studies on hydrazine derivatives in dogs (Palmer & Noel, 1963; Worden, Palmer, Noel & Mawdesley-Thomas, 1966; Noel, Worden & Palmer, 1967).

The routine list of tissues taken from dogs at HRC for subsequent histology is as follows:

- adrenal glands
- aorta (arch and abdominal)
- bone marrow (smear)
- brain (cerebral cortex, thalamic nuclei,
mid-brain, medulla, cerebellum)
- colon (2 sections)
- duodenum
- eye (including optic nerve)
- gall bladder
- gonads
- heart
- ileum
- jejunum
- kidneys
- liver

lungs
lymph nodes (cervical and mesenteric)
oesophagus
pancreas
pituitary gland
prostate gland
salivary gland
sciatic nerve
skeletal muscle
skin
spleen
stomach (fundus, body, antrum)
thyroid glands (including parathyroid glands)
thymus
trachea
urinary bladder
uterus

- and, when deemed necessary, the spinal cord.

The following are preserved but not routinely
processed:

bone
caecum
seminal vesicles
tongue

The above tissues are fixed in 10% buffered formalin for from 2 to 4 weeks, routinely processed in paraffin wax at 56°C, sectioned at 5µ, with wedge microtomes, and stained with haematoxylin and eosin. Despite the cautionary note sounded by Cameron & Cheng (1951) on the use of formaldehyde, the buffered formalin solution has given what appears to be satisfactory fixation. Automatic tissue processing is employed, using Technicon procedures, but all staining is done by hand since - to date - there does not appear to be any satisfactory means of automating haematoxylin and eosin staining for a variety of tissues such as those required for long-term toxicity studies.

In addition to the above, cryostat sections of liver and kidney fixed in formol calcium are cut at 12 μ and stained with Oil Red O for fat. Also, periodic acid-Schiff reactions are performed on liver and kidney to assess the glycogen content of the liver and the basement membrane integrity of the kidney.

Organ weight determinations are a routine feature of most long-term toxicity tests, although there is considerable controversy regarding their value and interpretation. The organs usually weighed are the liver, kidneys, heart, gonads, adrenal glands, spleen, brain, pituitary gland and, in the female, the uterus. Other organs or tissues may be added according to the type of material under test, e.g. the levator ani muscle in the case of steroids. It is customary to record actual organ weights and to express these also as a percentage of bodyweight.

Barnes & Denz (1954) queried whether increased organ weight alone could be accepted as evidence of disease and referred to Cameron (1952) in support of the view that many organs, including the liver and kidney, hypertrophy rapidly when given more work to do. 'The occurrence of hypertrophy is itself evidence of a healthy state of the organ and any associated histological damage should be reconsidered from this point of view. Certainly the hypertrophied tissues that have been reported in toxicity tests have not been shown

to function inefficiently and until they are, few pathologists are likely to accept an increase in weight as evidence of disease.'

Barron (1965) regarded organ weights as of little or no value, and Peck (1966), considering drugs, stated that such data were useful only if there was a specific effect on a target organ. Benitz (1970) says, however, that a specific effect may not be known at the time of autopsy. He considers organ weights and skeletal measurements together, and states that they provide the first morphological signs of dystrophic or dysplastic changes. 'Organ weight decreases usually indicate hypoplastic or atrophic changes: increased weights are more difficult to interpret. Hyperemia, hyperplasia, hypertrophy, enzyme induction phenomena and neoplastic changes play a role in this respect'.

Jackson & Cappiello (1964) recorded the ranges of normal organ weights in the Beagle, and concluded that - in nearly 80% of the organs examined - the histopathological diagnoses correlated with variation in organ weight.

In some investigations, the water content of the organs is determined by subtracting the weight after drying at 110°C for 72 hours from the wet weight. Benitz, Moraski & Cummings (1961) used this procedure indicating the results by numbers or by plus (+) signs. Mayer (1963) considered that + signs and grades in excess of 4 tended to complicate the presentation of the data. Wilcoxon (1945) indicated the possibility

of using ranking methods, i.e. methods in which scores 1,2,3 ... n are substituted for numerical data in order to obtain a rapid approximate idea of the significance of the differences observed in the experiment, and the procedure has been studied further by Wilcoxon (1947) and by Litchfield & Wilcoxon (1955). Benitz (1970) has given several examples of good correlation between the ranking of morphological changes and biochemical analyses.

An attempt was made by Chalkley (1943) to measure directly the volume or area ratios of components of tissues or organs under any conditions in which the various components are clearly distinguishable visually. He measured the hits recorded on nuclei, cytoplasm and other components (these last being designed collectively as tissue space), a hit being defined as the contact of the image of the point with the clearly observed image of the specimen. Point and line sampling techniques were subsequently reviewed by Hennig (1958), Weibel & Elias (1967) and Weibel, Kistler & Scherle (1966).

Benninghof (1950) showed that the nuclear volume of an organ could be helpful in assessing the functional state on the basis of swelling or contraction of nuclei, and the application and extension of such a procedure has been dealt with by Benitz (1969). Even though such measurements may be limited to determination of the largest and smallest diameter of a sufficient number of nuclei they, like the

procedures described above, are time consuming. What would appear to be a highly significant development, with extremely rapid counting, is the application of the image analyser, an instrument originally developed for metallurgy (Mawdesley-Thomas & Healey, 1969, a,b,c).

Golberg (1966) produced some interesting views on the possible interpretation of relative increase of liver weight in the rat by a study of various enzyme systems (see also Worden, 1970). The histochemical demonstration of enzyme systems is a field that has developed rapidly during recent years, and already by 1964 Pearse was able to review over 100 methods available for some 60 systems. 'More sensitive techniques are becoming available, and by their use and by better fixation it has been found possible to eradicate the diffuse colouration, due to the lyocomponent of hydrolytic enzymes, that formerly occurred. Enzymes may now be localized accurately within the cellular organelles, thus paving the way for effective cytochemical studies with the electron microscope.

At HRC, the following enzyme systems have been investigated with a view to their use in long-term toxicity and other studies:

- NAD diaphorase
- NADP diaphorase
- Succinic dehydrogenase
- Malate dehydrogenase
- Isocitrate dehydrogenase

Lactate dehydrogenase
 Glucose 6-phosphate dehydrogenase
 6-Phosphogluconate dehydrogenase
 Glutamate dehydrogenase
 Glucose dehydrogenase
 α -Glycerophosphate dehydrogenase
 β -Hydroxybutyrate dehydrogenase
 Uridine diphosphoglucose dehydrogenase
 Deoxyribonuclease
 Ribonuclease
 Cytochrome oxidase
 Aminopeptidase
 γ -Glutamyltranspeptidase
 N-Acetyl β -glucosaminidase
 Acid phosphatase
 Alkaline phosphatase
 Adenosine triphosphatase
 5-Nucleotidase
 Non-specific esterase
 Acetylcholinesterase
 β -Glucosidase
 β -Glucosidase
 β -Galactosidase
 β -Glucuronidase
 Glucose 6-phosphatase
 Phosphorylase
 Amylo 1,4 1,6 transglucosidase
 Phosphoglucomutase
 Uridine diphosphoglucose - glycogen transferase
 Aldolase
 Amylase

Histochemical techniques have been employed to help to distinguish between different forms of myocardial damage. Balusz & Jasmin (1964) reported that in primary cardiomyopathy (induced by plasmocid or by dihydro tachysterol) there were relatively slow changes in phosphorylase activity, and that these changes had an opposite gradient to that for glycogen reserves. In secondary cardiomyopathy (produced by coronary artery ligation and the injection of vasopressor substances) there was a rapid and sometimes complete loss of phosphorylase, with a parallel disappearance of stainable glycogen.

Although the electron microscope has been employed for biological investigations for many years - according to Harven (1967) it was first so used by Dr. L. Marton in 1934, while the writer collaborated with Dr. Egon Kodicek in 1942 over its use in a microbiological problem eventually solved by other means - it is mainly during the past decade that satisfactory accompanying preparative and staining techniques have been employed. These techniques and their application have been summarized by Harven, who has taken earlier and more extensive reviews into account, and their future use in experimental (and clinical) toxicology and in nutritional experiments would appear to hold considerable promise.

The application of electron microscopy to tissues taken from animals in subacute or long-term toxicity studies has been undertaken partly in order to obtain better definition of the resultant changes but also in the hope that the reliable early detection of characteristic 'lesions' might help to do away with prolonged and expensive investigations. Several such studies have been reported at meetings of the Society of Toxicology during recent years. Most of those have related to rats and monkeys, but Vernon, Homan & Tusing (1968) produced severe changes in the zona reticularis of the adrenal cortex of dogs by administering O,p'-DDD, i.e. 2-(o-chlorophenyl)-2-(p-chlorophenyl) 6-methyl pyrimidine for 15 days, and by electron microscopy showed that surviving cells contained large quantities of a dense material distorting an otherwise intact nucleus, and that there were greatly

reduced numbers of apparently normal mitochondria. There were variable findings, in relation to dose, in the mitochondria of the zona fasciculata.

The in vitro approach to the study of cell fractions has led to exciting discoveries that may well find their application in future to routine toxicological procedures. In 1955 C. deDuve (see deDuve, 1963) isolated from homogenized liver tissue by differential centrifugation a cell fraction that possessed powerful hydrolytic enzymes and contained numerous spherical bodies that were bounded by a single membrane and were without a characteristic internal structure. Under the heading 'Lysosomes - a pipe-dream come true', an article in Food and Cosmetic Toxicology (6, 779-780, 1968) says that: 'On the assumption that the lytic enzymes were derived from these bodies, they were called lysosomes and it was postulated that cell damage resulted from rupture of the limiting membrane with consequent diffusion of the enzymes. In the wake of this biochemical discovery, histochemical techniques were developed to identify the organelles and to demonstrate some of the complements of enzymes found biochemically.... The trivial name coined for these new organelles - "suicide bags" - soon became enshrined in the vocabulary of cell biologists, and directed scientific thought towards the acceptance of the lysosomes as some biological Valkyrie, coming on the scene during the terminal stages of cell life to complete its

ultimate dissolution. In vitro experiments using intact cells in tissue culture tended to confirm this early theory, since rupture of lysosomes was followed by irreversible injury and necrosis.'

Most of the studies reported to date have concerned rats or other smaller experimental animals, but similar procedures are likely to be applied to the dog, in which it may confidently be predicted that, e.g. watery vacuolation of the liver is due, as in other species, to autophagic vacuoles appearing at the site of local cell injury.

Fluorescent microscopy is another technique that would seem to be of potential value in the interpretation of toxicological findings (Worden, 1970, pp. 164-165), but to date it has been applied mainly to the tissues of species other than the dog.

At the present time, it must be concluded that careful, conventional histopathology, while it may usefully be supplemented by other techniques and by biochemical or metabolic investigations, remains a key feature of the interpretation of long-term toxicological or nutritional changes. Reference has already been made to the controversies that may arise between different pathologists, and this was again made evident in the paper by Nelson (1965) and the ensuing discussion. 'Acknowledged experts differ - often vociferously - on such points as whether slides should be read "blind" or by the person who has seen or supervised the

autopsy and is fully familiar with the whole background of the experiment. Nelson has shown how conventional data may best be summarized, but the need for a quantitative evaluation of those data capable of objective assessment is evident.' (Worden, 1970, p.417). The need for conventional examination is nevertheless fully justified, although such examinations should never be so routine as to exclude or to miss important findings, and this applies particularly to the central nervous system.

An example of the need for detailed study of the central nervous system of the dog was furnished by the series of studies undertaken at HRC and at the University of Cambridge on various hydrazine derivatives, using the Pembrokehire Corgi as the experimental animal and employing the techniques of Palmer (1965). These studies have been reported by Palmer & Noel (1963, 1965) and by Worden, Palmer, Noel & Mawdesley-Thomas (1966), and a copy of the paper by Worden et al. is appended to the thesis, since it illustrates the changes that were detected, including oedema of the cerebral white matter associated with glial proliferation.

4 : 9 Disease conditions and parasites that may
complicate the conduct or interpretation
of long-term experimental procedures

Most experimental dogs of a kind referred to in this thesis are vaccinated or given other forms of protective treatment for canine distemper, leptospirosis and the form of hepatitis originally designated 'Rubarth's disease'. They are also treated against helminth infestations and, in appropriate circumstances, against ectoparasites. In the U.K., precautionary measures are not taken against rabies, except for the strict procedures that are adopted to cover the entry into the country of dogs, cats and other animals from overseas - the precautions being tightened even further as a result of the final report of the Committee of Inquiry on Rabies, under the chairmanship of Mr. Ronald Waterhouse, Q.C., in July, 1971. Despite the fact that the U.K. appears to have the highest density of dogs and cats per head of human population, there have been 2 recorded cases of rabies only (in 1969 and 1970) since 1947, and indeed relative freedom for many years before that. Dogs are quarantined for 6 months on being brought into the country, but this has not hampered experimental work in appropriate circumstances. Thus, at HRC, a part of the premises was declared a quarantine station to enable work to be conducted on a Husky, prior to the British Trans-Antarctic Expedition (see Taylor, Worden & Waterhouse, 1958, 1959).

The many variants of the canine distemper complex, the position on which was summarized up to 1952 by Whitney & Whitney (1953) may cause considerable confusion within experimental dog colonies and their control requires constant vigilance and an up-to-date vaccination or prophylactic programme. At HRC during 1963 some untoward reactions were observed in a group of Corgi dogs that were at first attributed to the test material but, since they were found to be distributed among undosed controls as well as other dogs was recognized as being unrelated to the experimental procedure. The diagnosis of distemper was made only after several weeks of observation and investigation by medical and veterinary colleagues and consultants, but its detection led to a further reinforcement of the vaccination procedure, with the administration of a 'booster shot' following arrival at HRC itself.

HRC suffered also, in the early days, from an outbreak of Rubarth's disease that was hitherto unseen by those dealing with it. The numbers of dogs then maintained were few, and related to nutritional or metabolic studies only, and were of several breeds. A young male Dachshund succumbed overnight, with few if any detectable symptoms, a female 3-years-old Pyrenean Mountain Dog manifested neurological as well as hepatic disturbance and died after an illness lasting nearly 10 days, and a 2-years-old male Scottish Terrier recovered in the context of being able to survive for several years but was never again completely normal: it actually escaped and wandered, in apparently a dazed condition, and

was found next morning in a ditch by some common land where it and the other dogs were then normally taken daily for exercise. In the fatal cases, diagnosis was confirmed by the finding of typical hepatic cell lesions.

Worden, Noel & Jolly (1963) have summarized the position regarding leptospirosis as follows:

'Two forms of leptospirosis are known in the dog; both are transmitted in the urine of infected animals.

'Infected dogs are depressed, with a very tender abdomen, which is often so painful as to interfere with the gait of the animal. There is reddening of the conjunctiva and mouth, vomiting, varying degrees of diarrhoea and a marked loss of weight. There may be convulsions and a high fever. A thick discharge from the eyes is common, and the urine is scant and highly coloured.

'The more serious form of leptospirosis, due to infection with Leptospira icterohaemorrhagiae, is spread through the urine of infected rats, and this condition, associated with jaundice, can be transmitted to man. The other form of leptospirosis, in which jaundice is not a feature, is caused by Lepto. canicola and is spread from dog to dog. A vaccine against both forms of leptospirosis is now available.'

Reference has already been made in 4:3 to an unusual nematode infestation that occurred in a Beagle colony in Great Britain that had access to grass. The common round-

worms of the dog in the U.K., viz. Toxocara canis - which can arise from intrauterine infection (Morgan & Hawkins, 1949) - and Toxascaris leonina, are fairly easily controlled in an internal, or internal plus concrete run, environment by routine anthelmintic treatment and, fortunately, heartworm or Dirofilaria immitis infestation is not enzootic. It is, however, a major problem in some areas, particularly in the East and South, of the U.S.A., and McKelvie (1970a) has written: 'Heartworms are hazardous in Beagle colonies because of toxic or side effects in administering filaricides. In large colonies, microfilaria counts above 25 per ml may warrant euthanasia.'

Habermann, Fletcher, Williams & Thorp (1961) have reported that coccidiosis associated with Isospora species may be a serious problem in the U.S.A., especially that due to Isospora bigemina, which may produce a diffuse haemorrhage of the intestinal mucosa, but McKelvie (1970a) states that the condition may be satisfactorily treated, primarily with the enteric sulphonamides.

Ectoparasites are rarely a major problem in closed dog colonies in the U.K., although in some countries, e.g. Japan, stringent precautions have to be taken to prevent mosquitoes and other flying insects, which can transmit serious infections or infestations. As McKelvie (1970a), writing of the U.S.A., states: 'The most common ectoparasites are fleas, lice, ticks, and mites. In closed colonies these parasites as a

rule are not a problem once under control, and the usual antiparasitic dip is satisfactory (1% chlordane). As a continued precautionary measure the routine dipping of the entire colony at 6- or 12-month intervals has been found adequate to control these parasites.... Fleas can be more difficult to control than lice, as lice complete their life cycle on the host, whereas fleas deposit eggs in cracks and crevices.'

Of mange, McKelvie (1970a) writes: 'The more common mange mites in the dog include sarcoptic, demodectic and otodectic species. Of these, Demodex folliculorum is probably the most serious kennel problem because these mites have been found as a facultative pathogen in about 80 percent of dogs [Greve & Gaatar, 1964]. Otodectic mites can be a serious problem if neglected, but these parasites are easily controlled by a bland oil containing a nonirritating insecticide. If several Beagles are observed infested with ear mites, it is advisable to treat the entire colony. Severe otitis externa caused by mites may require daily cleaning with special medicants.'

Although not recorded as a problem in laboratory dogs in the U.K., the increased incidence of Cheyletiella parasitovorax infestation in domestic dogs has recently been noted by Smith (1971). None of 24 affected dogs of which he writes has manifested any sign of pruritus, but one owner herself

showed generalised papular dermatitis with pruritus. 'The usual case presented in the surgery is one of generalised scurf and dryness of the coat. Young puppies can be very much affected, but the adults in close contact with them can be relatively clean.... The mites are very susceptible to change in temperature and die very quickly if parted from the host.' Gamma benzene hexachloride appears to be the treatment of choice.

McKelvie (1970a) states that: 'Fungal infections can occur in Beagles and the most common is ringworm (Microsporum canis). A tentative diagnosis of M. canis infection can be made by an ultraviolet light (Wood's lamp), but direct microscopic examination and culture are required for positive identification. For treatment Foley [1961] suggests the oral administration of griseofulvin and the topical application of an aerosol containing undecylenic acid.'

Worden et al. (1963) referred to impacted anal glands as follows:

'The dog carries two anal glands, the ducts of which enter the anal rim. These sacs produce a secretion which ranges from a grey paste to brown liquid. Due to an apparent inability of the gland to empty itself or through blockage of the ducts, the anal glands become distended and in its attempts to alleviate the discomfort the dog will drag its anus along the ground. The glands can be emptied by placing

one finger (preferably gloved) inside the anus and squeezing with the thumb outside. If the glands do not easily empty with reasonable pressure, excision of the gland may be necessary.'

Of toe nails, the same authors wrote:

'Overgrown or malformed toe nails are sometimes observed, especially in dogs that do not obtain much exercise. Such nails need clipping. Care should be taken to avoid the sensitive core, which can be seen if the claw is carefully examined. It is usual to cut about $\frac{1}{4}$ in (6 mm) distal to the core.'

Brucellosis has not been regarded as a likely problem among laboratory dogs, but Gleiser, Sheldon, Van Hoosier & Hill (1971) have recently described a spontaneous outbreak, characterized by abortion, that occurred between 1964 and 1967 in a dog colony maintained at the Baylor College of Medicine, Huntsville, Texas. The principal pathological lesions were found in the lymphoid tissues, the principal and accessory male sex organs, and the renal glomeruli. An organism (Brucella canis) was isolated from the spleen in a **dog** that did not have a bacteraemia.

Gething (1971) has described an abnormal skin fragility in a 10 $\frac{1}{2}$ -years-old male Beagle that appeared to bear a marked resemblance to the Ehlers-Danlos syndrome in man. 'The skin was of normal thickness, but showed numerous scars

over the entire body surface. Hair growth appeared normal except over the scars. The skin was excessively pendulous under the chin, neck and trunk, and lay in folds over the legs and head. Close examination of the integument revealed hyperlaxity, but no hyperelasticity; the skin could be easily stretched from its normal position but did not recoil when released. Skin sensation appeared normal and there was no evidence of pseudotumours, subcutaneous nodules or joint abnormalities.

'The dog was not known to have previously received corticosteroids or other drugs which could have affected the skin.'

A diffuse cellular infiltrate was scattered throughout the dermis, which contained prominent thin-walled blood vessels. The epidermis was only 1 to 3 cells thick and contained a number of balloon cells, with no evidence of a prickle cell layer.

Among the more difficult problems facing the experimental toxicologist is that of predicting hypersensitivity reactions or so-called allergic reactions in man. The natural occurrence of hypersensitivity conditions in the dog has often been alluded to (see Worden, 1951), and Halliwell & Schwarzman (1971) have now brought forward more positive evidence of the existence in this species of atopic disease, in the sense defined by Coca & Cooke (1923), i.e. the clinical entities of asthma, hay-fever and a dermatitis primarily

affecting children. In the dog, however, the condition is primarily one of pruritus, and Halliwell & Schwartzman (loc.cit.) refer to 60 cases seen over a 2-year period among a total of 14,379 canine patients seen, with a highest breed incidence among Wire-haired Fox Terriers but with some cases also in Scottish Terriers, Cocker Spaniels and Dalmations, of those referred to in this thesis, as well as in West Highland White Terriers, Sealyham Terriers, Dachs-hunds, German Shepherd Dogs (Alsations) and other breeds. Some evidence of reaction to allergenic extracts was obtained, particularly with ragweed.

Wilkinson & Prydie (1971) have recently reported possible cases of the ovine disease, 'orf' or contagious pustular dermatitis (also known as contagious acthyma) in a pack of Fox Hounds, but this seems an unlikely new hazard to laboratory dogs.

Renal disease has long been recognised as a common naturally-occurring condition in the dog (see 3:6), and reference has already been made to the long-term study of Richards & Hoe (1967), who have shown that there is an over-all trend towards metabolic acidosis in nephritic dogs. The routine laboratory procedures adopted in experimental colonies are usually sufficient to detect spontaneous cases of renal disease, but the attitude of the investigator towards it must be one of constant vigilance, especially in the case of experiments that may last for a year or longer.

Borthwick & Mackenzie (1971) have recently reviewed prostatic disease in the dog, and quote earlier authors, including Archibald (1965), who have concluded that most entire male dogs over the age of 5 years have some degree of prostatic hypertrophy. The condition may interfere with the passing of a catheter and diagnostic procedures include digital rectal examination, manual palpation of the abdomen, plain radiography and the taking of pneumocystograms. The importance of recognizing the spontaneous nature of this disease in the case of long-term experiments is obvious.

Neoplastic disease in dogs, with special reference to the Beagle, has already been discussed in 3:6, where attention has been drawn to mammary tumours. These may occur spontaneously in other breeds that are used for experimental purposes, however, and Proud & Preece (1971), e.g., refer to the therapeutic measures adopted in a 13-years-old Corgi presenting with a multiple mammary tumour large enough to restrict movement. With the possibility of virtual life-time studies in dogs for hormonal preparations, there is a need to differentiate such tumours from those possibly attributable to the test substance or formulation.

5 : CONCLUSIONS AND RECOMMENDATIONS

5 : 1 The value of the dog as an experimental animal

An example of the use of the dog in a typical long-term study at HRC is given in Appendix V, which represents a manuscript by the author and his colleagues (Worden, Noel & Mawdesley-Thomas, 1972), of which a shortened version (without much of the tabular material) is in press in Toxicology and Applied Pharmacology. Similar examples were given in detail in Worden (1970), while the toxicity of telodrin, conducted by somewhat earlier procedures, was published, with information based on the dog and on the rat, by Worden (1969). It may safely be assumed from the way in which these examples, and the many other experiments conducted at HRC and elsewhere have been accepted by the authorities in different countries, that the dog is a standard 'non rodent' with the widest current use in long-term toxicity studies. Many examples have been given or cited in this thesis which, added to the long history of the species as a test animal in the nutritional field, indicate that its current and future value in long-term work is not confined solely to toxicology and to predictive safety evaluation studies.

The features that help to establish the value of the dog include its availability, tractability, relative degree

of homogeneity (provided purebred strains are employed) and relative ease of maintenance as a healthy animal. Of equal importance may be included the considerable background of information on the normal dog and the acquaintance of veterinarians with the anatomy, physiology, diseases, parasites, abnormalities and clinical examination of the species.

The different breeds vary in their behaviour and in their capacity to live together (Worden, 1959), but the most commonly employed, the Beagle, is docile and has the greatest amount of background information available. Its original choice appears to have been based as much as anything else upon the availability in sufficient numbers, but despite the existence of certain heritable anomalies - a factor that would probably be operative in any other breed used and studied to the same degree - its contribution has been invaluable.

It is a fascinating coincidence that the name Beagle was associated with some of the experiences that led to the foundation, through Charles Darwin,¹ of some of the most important concepts of modern biology, when he accepted the invitation of Captain Fitzroy to circumnavigate the world, without pay, as naturalist in H.M.S. Beagle. 'The voyage of the Beagle' said Darwin (1845) 'has been the most important event of my life and has determined my whole career.'

For reasons such as the metabolic pathway or rate of metabolism of a given test compound, a non rodent other than

¹See also Moorehead (1969)

the dog may be preferable for certain studies. Again, the dog is sometimes more sensitive than other species, including man himself, to other classes of compound. This is probably the case with hydrazine monoamine oxidase inhibitors and related hydrazine derivatives such as isonicotinic acid hydrazide, or isoniazid, which Noel, Worden & Palmer (1967) reported as having neuropathological effects in the dog that had not been detected in rats (Harper & Worden, 1966) and have not been reported clinically in man, despite the widespread use of this compound over many years in the treatment of human cases of tuberculosis. Indeed it is interesting to speculate whether or not isoniazid would have been introduced into therapeutic use had intensive long-term dog studies been required at the time it was being developed. Despite such possibilities, however, it must be appreciated that a greater sensitivity than that of man may exist in the case of other compounds in other species, and that the future approval or rejection of a test material is unlikely to be determined on this basis alone.

Boyd (1968) put forward a plea for the use of the dog in predictive drug toxicity on the grounds that it possesses a projectile vomiting reflex and is therefore suited to the investigation of possible emetic action.

There are instances in which the dog may differ so markedly from man in its physiology or its reaction that the use of another species would seem to be preferable. At

HRC, the author and his colleagues were never very happy in the use of the dog as a test animal for evaluating the irritability and toxicity of spermatocidal formulations or other materials introduced intravaginally. The oestrus cycle in the dog, and the accompanying changes in the vulvo-vaginal mucosa, differ so markedly from those of the human menstrual cycle that the danger of quite unrelated findings seemed to be considerable. On the other hand the long-term toxicity of progestational steroids and oral contraceptive preparations in the dog appear to be a rational procedure, especially as the near-lifetime studies under way are being compared with investigations of even longer duration in the rhesus monkey, which has of course a menstrual cycle.

5 : 2 The future pattern of long-term animal
experimentation

The present author concluded from his studies on the toxicity of compounds of veterinary and agricultural importance (Worden, 1970) that despite the many changes that had occurred during the previous decade the general pattern of testing remained very much the same '... and despite some exciting developments at the cellular and subcellular levels of investigation, and many refinements in the examination of the intact animal, it seems probable that it will continue for some years at least - and possibly for longer. What may help to promote more rapid or more convincing use of animal studies, or of in vitro systems, is the series of advances that are being made in analytical chemistry. These, combined with the use of enzyme histochemistry, electron microscopy or fluorescence microscopy should enable much more thorough quantitative assessments of toxicity. Almost every technique, however, indicates the existence of new problems as well as helping to solve known ones. The complete substitution of in vitro systems for the intact animal model, however imperfect the latter may be in terms of predicting the hazard to man, is as yet a long way off.'

This assessment seems to be shared by, or to be in agreement with the conclusions of, other authors who have attempted to review the status of toxicology, or of some

important aspect of it, including Abrams, Zbinden & Bagdon (1965), Boyd (1965), Golberg (1965, 1966), Beyer (1966), Janko (1966), Mihich (1966), Peck (1966), Ruckebusch (1966), Boyland & Goulding (1968), D'Agnanno (1968), Goldenthal (1968), Loomis (1968), Frazer & Sharratt (1969) and Benitz (1970). Even Golberg (1966), who has been concerned with pioneer work in the development of new techniques and their adaptation to toxicology, has emphasized the lack of change in basic approach to the overall form of testing, while in her capacity as Chairman of the HRC Scientific Advisory Council Dame Honor Fell, whose work on tissue culture extends back over more than 40 years, has repeatedly emphasized that the techniques must not be accepted as a substitute for whole-animal investigations.

The author's colleague Rofe (1971) has pointed out that, despite the long period over which tissue culture has been employed, there does not appear to have been any systematic comparison of the reaction of normal adult human tissues with animal tissues in toxicological studies, and has offered proposals for research in which a selection of animal tissues would be compared with human tissue in their response to substances for which substantial data from in vivo toxicology already exist. Only when such research has been undertaken will it perhaps be possible to contemplate a 'screening technique' based upon tissue culture. Nor would such a technique necessarily preclude subsequent confirmatory long-term animal tests.

There is indeed an analogy with the use of image analysis, with suitable computerization of the data obtained, of sebaceous gland suppression as a screening technique for carcinogenic potential. Work at HRC, largely unpublished, has indicated that the suppression of sebaceous gland activity, by a variety of compounds including many known carcinogens, may be measured rapidly and actively by the modern image analyser, hitherto employed in metallurgy. The system is now sufficiently well advanced to be employed as a routine screen for comparing different tobacco condensates, the routine carcinogenic hazard of which has hitherto been comparable on the basis of skin-painting tests in mice lasting up to 18 months or more. The degree of correlation with 'known' carcinogens is excellent, but there can be 'false positive' results, e.g. pyrene, which is not recognized as a carcinogen and which so far as is known is not transformed into a carcinogen in vivo, suppresses sebaceous gland activity in much the same way as the known carcinogen 3:4 benzpyrene. In practice, a short-term in vitro or in vivo test for toxicity or carcinogenicity would, at the present time, exclude the admission of many candidate materials from long-term study, or would cause them to be examined even more closely than would otherwise have been the case, e.g. by employing more species for the long-term testing.

The author is very firmly of the opinion that future long-term tests, whatever the nature of the test compound,

should include a 'recovery period', i.e. a period following completion of the test procedure in which some of the animals are maintained on normal diets before eventual sacrifice and pathological examination. The longer the duration of the experiment, the greater the opportunities for the development of non-specific or gerontological changes in the tissues. The more that is known of the background to a species, the greater the appreciation of the existence of heritable defects or of physiological changes-with-age that may be confused with histological, histochemical or biochemical lesions. There would seem therefore to be ample reason to allow the pathologist in particular the opportunity to study internal control material based upon tissues, body fluids or enzyme systems that have been left unexposed for a period of some weeks or more to the test substance. This would seem to be valid for the changes occurring not only in toxicology studies but also in those that result from nutritional deficiency or other procedures calculated to induce detectable variations in body structure or composition.

As has already been emphasized earlier in this thesis, the future of long-term animal investigations that relate to predictive human evaluation is likely to depend to an increasing extent upon comparative metabolic data, including the early exposure of man himself to small doses of the test material. Such data will add to the value of the animal study, and the general conclusion must be that the long-term animal study is likely to remain a cardinal feature of

experimental toxicology, and of certain other branches of medical or veterinary biology, for several decades to come. There will be refinements in the nature of the test animals themselves, even in the case of the dog, and this will yield information that is of value to veterinary medicine as well as to experimental science.

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APPENDIX I

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PROCEEDINGS OF THE EUROPEAN SOCIETY
FOR THE STUDY OF DRUG TOXICITY

VOLUME VIII, NEUROTOXICITY OF DRUGS

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LESIONS IN THE BRAIN OF THE DOG INDUCED BY PROLONGED ADMINISTRATION OF MONO-AMINE OXIDASE INHIBITORS AND ISONIAZID

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In this paper we describe toxic damage produced in the central nervous system of dogs treated with mono-amine oxidase inhibitors and with isoniazid and its derivative. The toxicity studies on a series of new hydrazine mono-amine oxidase inhibitors (MAOI's) were performed between 1960 and 1962.

MONO-AMINE OXIDASE INHIBITORS

Methods

Groups of dogs (Pembrokehire Corgis) were dosed orally with these compounds at various levels. Negative undosed animals were used together with positive control animals. The latter were dosed with the various parent MAOI compounds (Fig. 1). Other groups of animals were dosed with newer MAOI

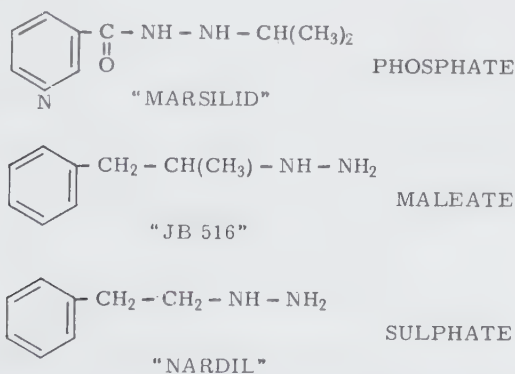


Fig. 1. — Parent mono-amine oxidase inhibitors.

compounds in which the hydrazine moiety was directly linked to the organic nucleus (Fig. 2). Finally, other groups of animals were dosed with MAOI compounds in which the hydrazine moiety was separated from the nucleus by an oxygen link (Fig. 3). No attempt has been made in this paper to com-

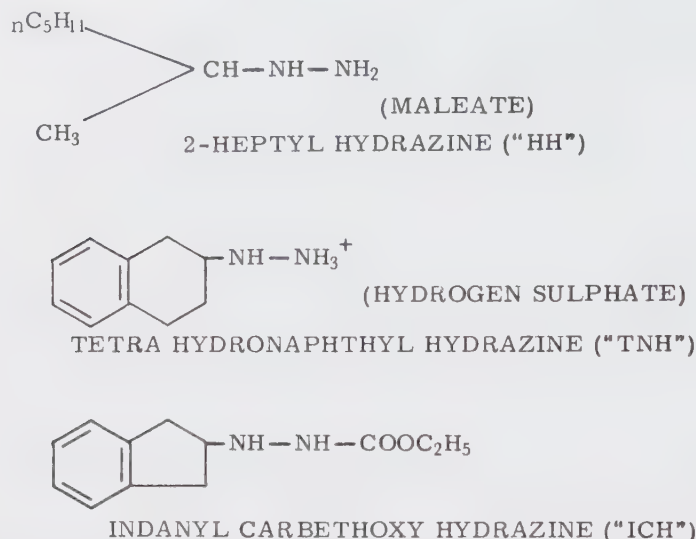


Fig. 2. — Mono-amine oxidase inhibitors with hydrazine moiety directly linked to organic nucleus.

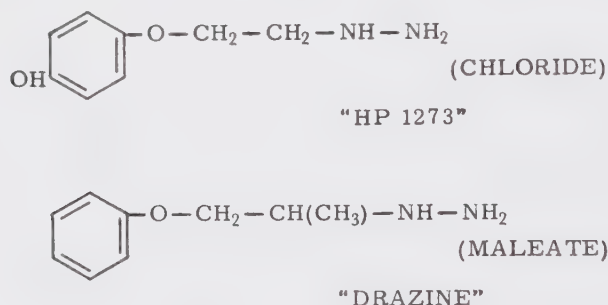


Fig. 3. — Mono-amine oxidase inhibitors with hydrazine moiety separated from the nucleus by an oxygen atom.

pare relative toxicities within groups. These studies have previously been recorded by two of the authors (Palmer and Noel, 1963 and 1965). Later investigations were undertaken on the toxicity of isoniazid and its methanesulphonate derivative (Fig. 4). Many of these drugs caused neurological signs and pathological changes in the brain.

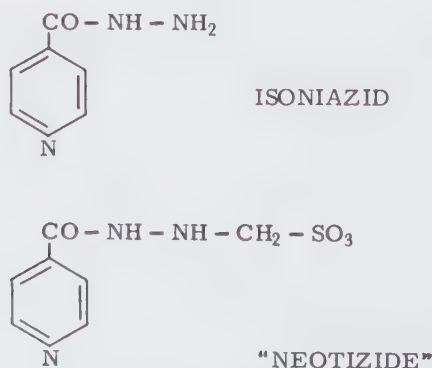


Fig. 4. — Isoniazid and its methanesulphonate derivative.

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Clinical observations

The compounds under test could be divided into three groups according to the clinical signs indicating toxic changes; these are listed in Fig. 5. In each

	Haemolytic anaemia	CNS stimulation	Cerebellar ataxia	Convulsions
Marsilid	+++	—	—	—
"JB 516"	+	+	+	Inconsistent late
Nardil	+	+	+	+
"HH"	+	+	+	—
"TNH"	+	+	+	rare
"ICH"	+	+	+	+
"HP"	+	+	—	+
Drazine	+	+	—	+

Fig. 5. — Toxic symptoms caused by the various monoamine oxidase inhibitors.

compound there was usually a relationship between the dose level, the rate of development of signs and also the interval before the onset of signs. Correlation between the signs and the histopathological findings was of the utmost interest and importance.

Group 1

Marsilid was unique in that the only symptom observed was a haemolytic anaemia of some severity. At high dose levels rapid destruction of the entire peripheral red cell population occurred with resulting jaundice, gangrene of extremities and death. Recovery could be equally rapid if the drug was withdrawn, no neurological symptoms were seen and, unfortunately, no neuropathological examination was performed. It is interesting to note that in all the remaining drugs tested, a mild to moderate degree of haemolytic anaemia occurred, especially in the high dose levels. This appeared to be persistent but not progressive. Since all the compounds were hydrazines it is tempting to relate the haemolytic activity to this factor, especially as the non-MAOI compound, phenyl hydrazine, has been used clinically for this purpose.

Group 2

This group consisted of JB 516, phenelzine and the three compounds abbreviated to 'HH', 'TNH' and 'ICH'. These drugs all produced cerebellar ataxia, together with some central nervous stimulation which on relatively rare occasions resulted in convulsions. The cerebellar ataxia could lead on to death but could also result in a remarkable recovery if the drug was withdrawn.

Group 3

This group consisted of HP 1273 and phenoxypropazine both of which produced convulsions but no cerebellar ataxia. The compounds in this group have an oxygen atom or ether linkage joining the organic nucleus to the hydrazine moiety.

Excluding Marsilid from further discussion the other seven compounds could be divided clinically according to the presence or absence of cerebellar

ataxia. The group showing the signs of cerebellar ataxia also had neuropathological lesions in the brain stem or cerebellum or cerebral hemisphere. The two compounds in group 3, those having an ether linkage between the aromatic nucleus and hydrazine side chain, showed no evidence of cerebellar ataxia. Neuropathological examination of the central nervous system showed no evidence of damage in the animals dosed with phenoxypropazine (the material from animals dosed with HP 1273 was not available for histological examination). With the group of five compounds showing cerebellar ataxia and neuropathological change, it was possible to differentiate further between the compounds. With compounds JB 516 and TNH, cerebellar ataxia was a prominent and early sign, whereas convulsions were late and inconsistent. In these animals severe brain damage was seen in the inferior olives, a spongy state with gliosis was present in the cerebellum and microcavitation was occasionally seen in the subthalamic nucleus. By contrast, after dosing with phenelzine and ICH, convulsions were a prominent and early symptom and cerebellar ataxia tended to follow and become progressively worse. With these compounds, it appeared that brain damage was mainly in the subcortical white matter and although there were changes in the cerebellum no evidence of damage was seen in the inferior olives.

Neuropathology

JB 516

The main pathological features with this compound were seen in the inferior olive and in the cerebellum. In both inferior olives there is local thinning of the background material of part of the nucleus (Fig. 6). The first changes are spongy in appearance associated with some vascular hyperplasia. The

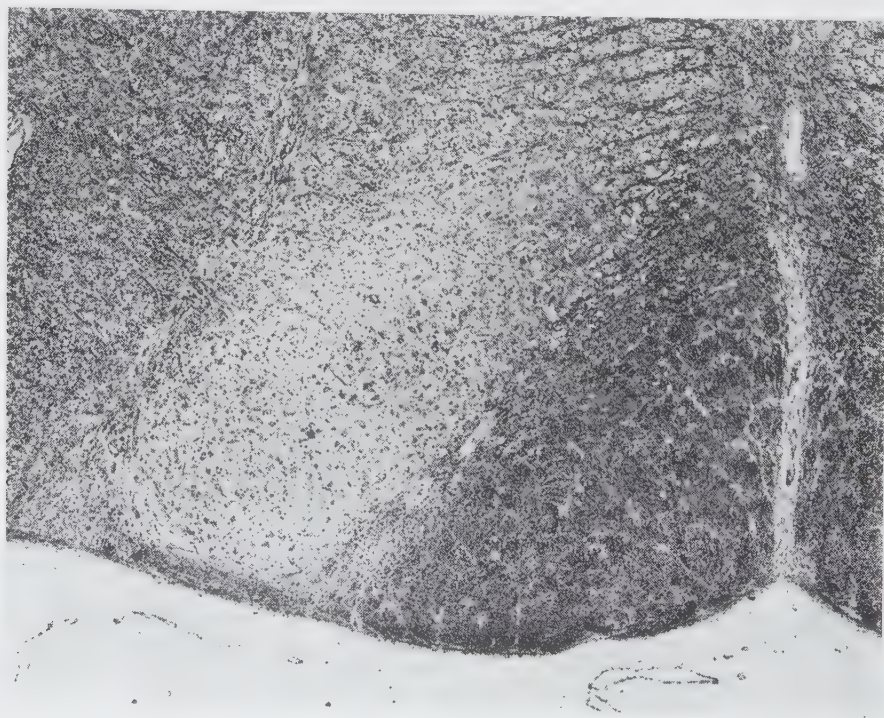


Fig. 6. — JB 516. 10 mg/kg/day for 59 days. Focal destruction of inferior olive. Methasol fast blue 2G. Mag. x 30.

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spongy appearance becomes exaggerated and there is loss of nerve cells, breakdown of myelin, production of lipid phagocytes and capillary proliferation. Changes in the cerebellar roof nuclei are also present; in the lateral part there is evidence of a slight spongy state associated with nerve cell loss, vascular hyperplasia and some proliferation of astrocytes.

Tetra-hydro-naphthyl hydrazine - 'TNH'

Neuropathological effects of this drug are very similar to those of JB 516 in that the inferior olivary nuclei are usually severely damaged (Figs. 7 and 8). Changes are also present in the cerebellum (Fig. 9). In the olives there is evidence of a profound hyperplasia of the smaller blood vessels with proliferation of endothelial cells so that the walls of the capillaries appear to be two endothelial cells thick (Fig. 10). The tissue surrounding the affected

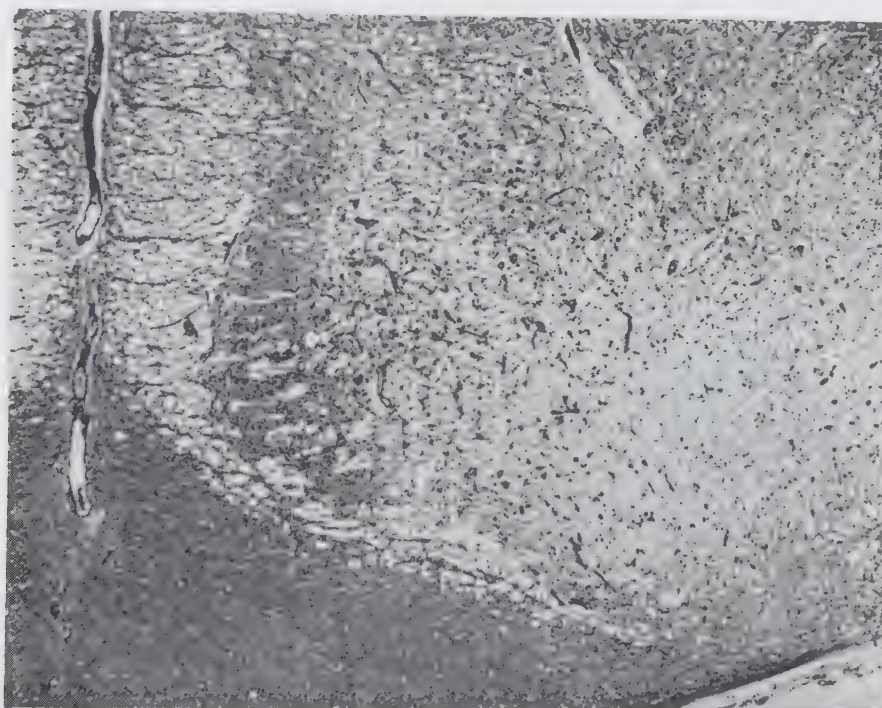


Fig. 7. — TNH. 3 mg/kg/day for 104 days. Severe malacia extending beyond the territory of the inferior olive. H & E. Mag. x 30.

olives is, to a great extent, normal although some minimal cavitation and astrocytosis is present. Neurons in the affected olives are fewer in number than normal, but despite the severe damage there are often healthy nerve cells in the centre of an area showing signs of severe malacia. In the lateral parts of the cerebellar roof nuclei an area of status spongiosus is seen in which there is an increase in microglia and some astrocytic proliferation. The microcavitation is often accompanied by gliosis which becomes more severe at the high dose levels. It is interesting to note that there is no direct relation between the dose level and the severity of the olivary lesions but that the severest changes occurred in dogs at a minimal dose level over a prolonged period.



Fig. 8. — TNH. 3 mg/kg/day for 140 days. Early status spongiosus in the inferior olive.
H & E. Mag. x 30.



Fig. 9. — TNH. 3 mg/kg/day for 104 days. Status spongiosus in the roof of the cerebellum.
Methasol fast blue 2 G. Mag. x 15.

Phenelzine

The most striking abnormality with this compound is in the cerebellum which shows evidence of bilateral ischaemic necrosis of the cerebellar roof

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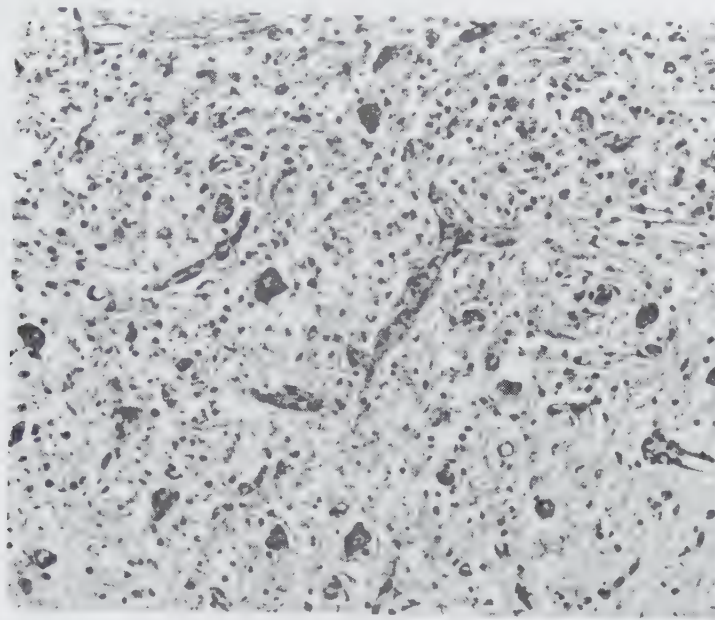


Fig. 10. — TNH. 5 mg/kg/day for 48 days. Inferior olive showing early vascular hyperplasia, microglial reaction and some neuronal loss. H. & E. Mag. x 160.

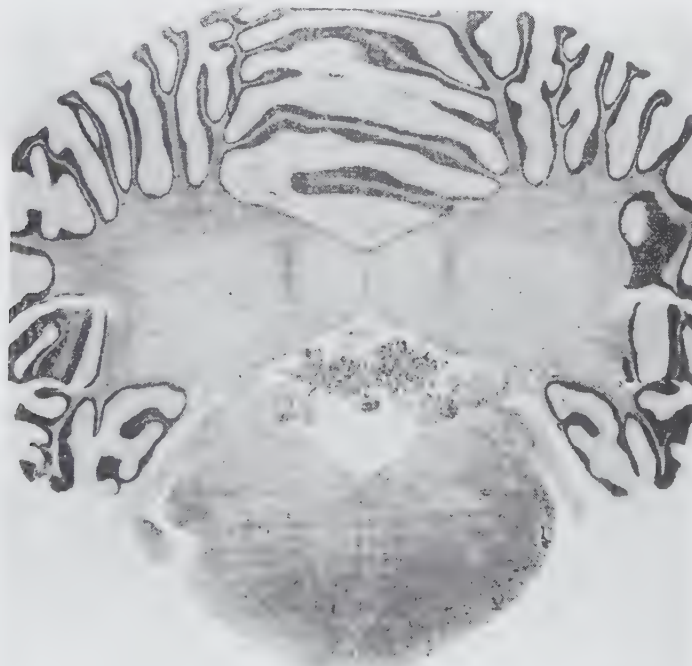


Fig. 11. — Phenelzine. 12 mg/kg/day for 12 weeks. Malacia of cerebellar roof nuclei. Celloidin. H & E. Mag. *c* x 4.

nuclei (Fig. 11). Examination of the brain stem including the inferior olives showed no abnormality. There is evidence of severe damage of the cerebral white matter (Fig. 12) as shown by the presence of microcavitation, microglial proliferation and the presence of pathological astrocytes.

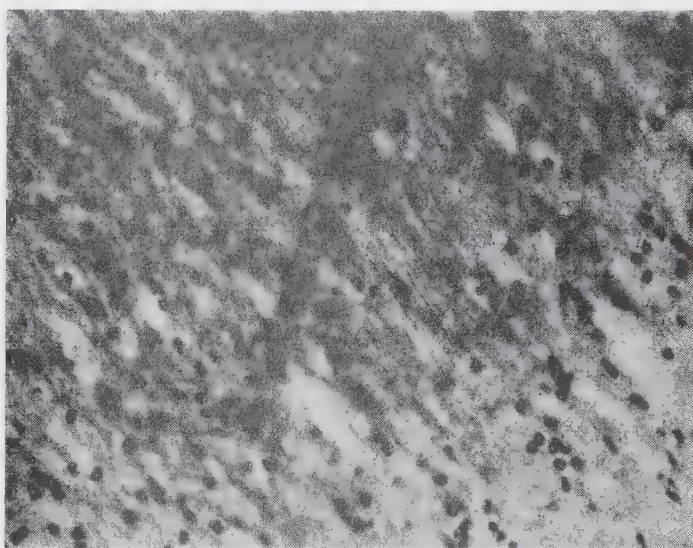


Fig. 12. — Phenelzine. 12 mg/kg/day for 12 weeks. Degeneration of cerebral myelin with microglia and capillary reaction. Celloidin, H & E. Mag. x 290.

Indanyl carbethoxy hydrazine - 'ICH'

The main neuropathological feature with this drug consists of separation of the myelinated fibres in the cerebral white matter and degenerative changes in the myelin (Fig. 13). The picture is compatible with status spongiosus. A similar picture is seen in the cerebellar roof nuclei. The brain stem in all animals examined was within normal limits.

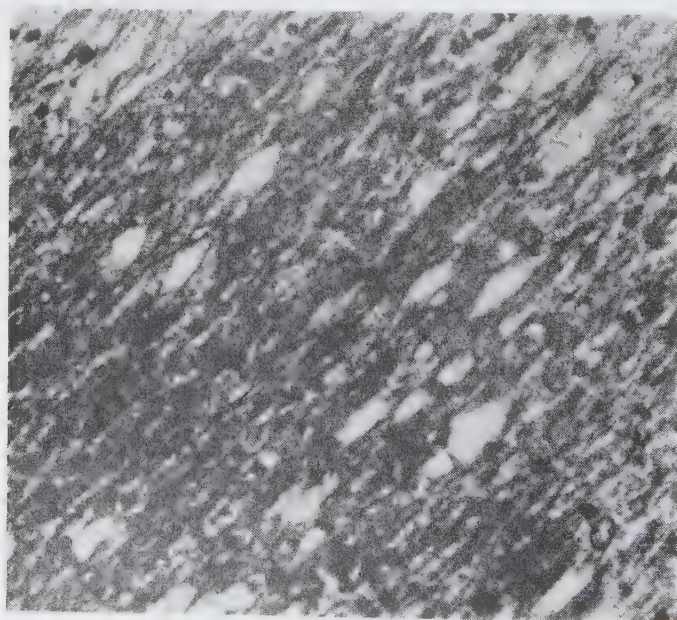


Fig. 13. — ICH. 2 mg/kg/day for 142 days. Early disintegration of cerebral myelin, with glial proliferation. H & E. Mag. x 290.

MONO-AMINE OXIDASE INHIBITORS AND ISONIAZID

Heptyl hydrazine - 'HH'

Neuropathological changes in these animals are minimal. There is gliosis in the roof of the cerebellum and vacuolation of certain neuronal groups (Fig. 14).

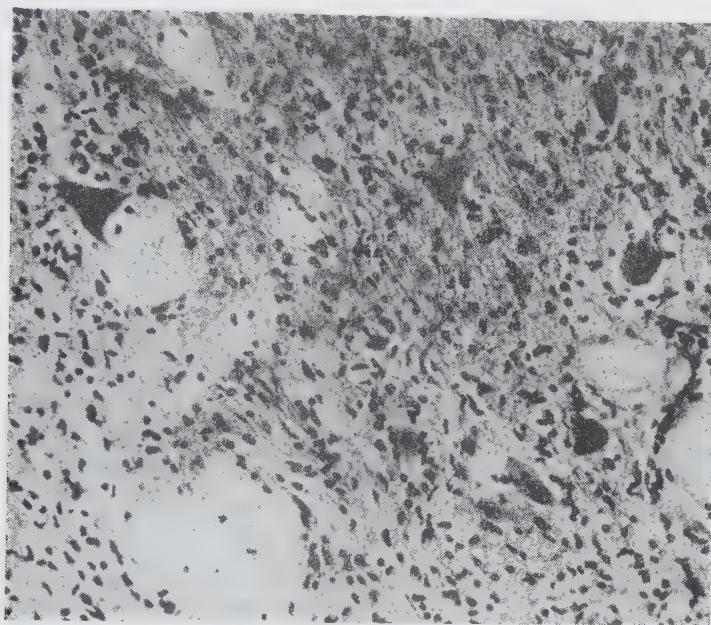


Fig. 14. — Heptyl hydrazine, 10 mg/kg/day for 12 days. Gross vacuolation of neurons in the lateral vestibular nucleus with severe microglial reaction. Celloidin. H & E. Mag. x 190.

DISCUSSION

At the time these experiments were completed Maling, Highman and Spector (1962) and Highman and Maling (1962) demonstrated pathological lesions in the inferior olive and also the pyriform lobe of dogs dosed with JB 516. Their animals had received the compound by subcutaneous injection whereas our animals were dosed orally. It is possible, therefore, that the absence of pyriform lesions in our animals receiving JB 516 could be accounted for by the difference of the route of administration. No liver changes were noted in any of our animals nor was any testicular damage seen although this, in all probability, was due to the immaturity of the animals used. At that time it seemed unlikely that the brain lesions found were related to the inhibition of mono-amine oxidase by these drugs.

The function of the mono-amine oxidase inhibitors is to allow the accumulation of the substrates of mono-amine oxidase. Of these, adrenalin and noradrenalin are unlikely to be of much importance since other routes for their destruction are available (Axelrod, Senoh and Witkop, 1958). In the experiments of Maling *et al.* (1962) noradrenalin levels were not elevated in the brain stems of dogs treated with these compounds although Uchida and O'Brien (1964a) have shown some slight increase in noradrenalin levels in rats. Corne and Graham (1957) showed that serotonin is the chief substrate for mono-amine oxidase activity. Mono-amine oxidase inhibition in the intact animal results in the elevation of serotonin in the central nervous system of both dog and rat (Maling *et al.*, Green *et al.*, 1962). It is known that high

concentrations of serotonin within the dog brain are strictly localised to grey matter being maximal in the hypothalamus, caudate nucleus, area postrema and mid-brain. Low concentrations are present in other areas of grey matter but little or none is found in the white matter and the cerebellum. The brain lesions seen in these experimental animals have therefore occurred at sites where serotonin is not normally found.

Serotonin is thought to have a local vasoconstrictor action on the brains of dogs (Kaneko, McCubbin and Page, 1960). An increase in pulmonary artery pressure occurs in dogs after the injection of serotonin and has been ascribed to constriction of pre-capillary vessels or of the pulmonary vein (Gilbert and Hinshaw, 1958; Shepherd *et al.*, 1959; Aviado, 1960). Kabins, Molina and Katz (1959) reported that in dogs serotonin also gives rise to pulmonary oedema, a feature frequently seen in the present experiments and it is suggested that this is due to an altered capillary permeability. Vasoconstrictor action of serotonin in the brain with perhaps associated oedema might therefore have accounted for lesions that occurred in the present experiments. The distribution of the enzyme mono-amine oxidase, within the central nervous system, is not known with certainty. It appears to exist in higher concentrations in those sites where serotonin is produced, but lower concentrations are present elsewhere. Since the observed neuropathological lesions seen in our dogs were at sites where serotonin was not normally found, it was postulated by one of us (P.R.B.N.) that where serotonin accumulated, it diffused into the surrounding areas that were not normally exposed to its action or protected from it. Although this hypothesis was attractive initially it now seems unlikely in the light of subsequent work performed by ourselves and other workers. The exact pathogenesis of these lesions is, as yet, unknown, and in fact may be unrelated to MAOI activity. An alteration in the brain glutamic decarboxylase has been demonstrated by Uchida and O'Brien (1964b).

ISONIAZID AND ITS METHANOSULPHONATE DERIVATIVE (NEOTIZIDE)

In 1963 we performed toxicity studies on isoniazid and its methanosulphonate derivative (Palmer and Noel, 1965). Neither of these compounds are MAOI's although they are hydrazides. They are both widely used in man as antitubercular compounds. In the dog, high doses produce convulsions but not cerebellar ataxia. Slight anaemia is present. The neuropathological effects of these two compounds are similar in that at high dose levels separation of fibres in certain areas of the subcortical myelin are seen. Clefts or spaces occur accompanied by activation of the microglia and there is hypertrophy of the astrocytes (Figs. 15 and 16). In stained sections there is pallor of affected myelin and occasional ballooning of myelin sheaths suggestive of early degenerative change. However, myelin breakdown products are not present. The axons remain intact and the majority of cortical neurons appear healthy although there is microcavitation of the ground substance which is probably oedema. The neuropathological effects are similar in these two compounds, although not at the same dose levels (Noel and Palmer, 1966).

DISCUSSION

The effect of isoniazid and Neotizide on the brain of the dog appears to be the production of oedema of the cerebral white matter associated with glial proliferation. The changes are very similar to those produced by some of the

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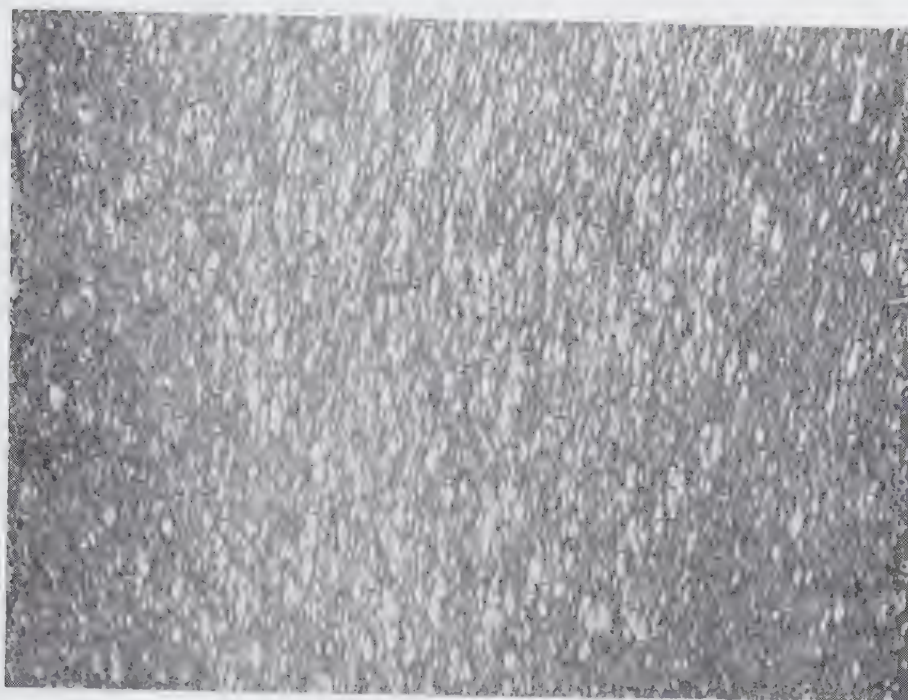


Fig. 15. — Isoniazid 25 mg. reduced to 15 mg/kg/day for 113 days. Central white matter showing severe separation of fibres. H & E. Mag. x 38.

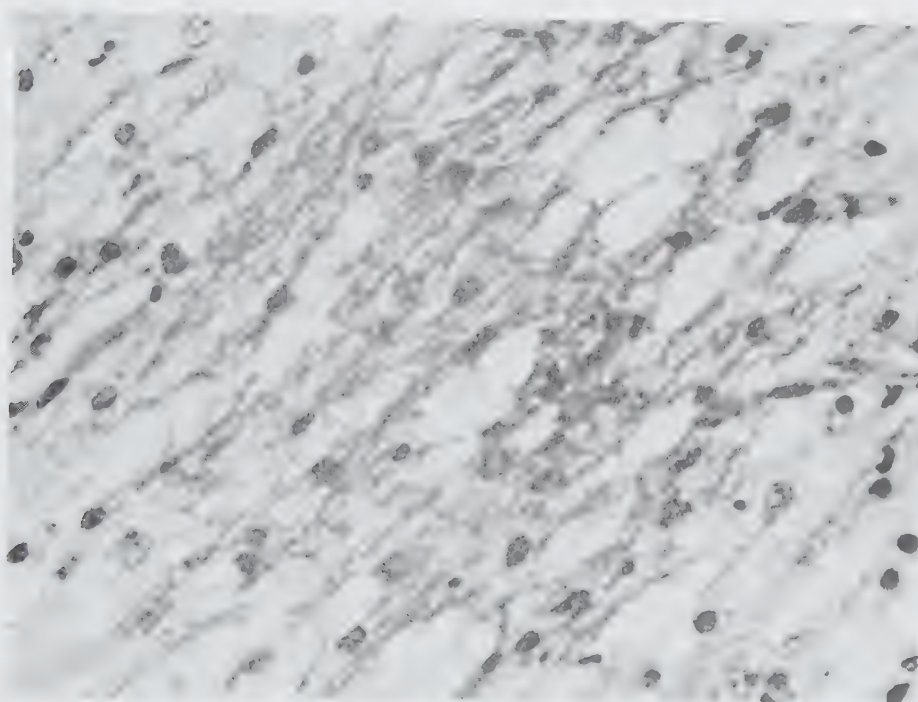


Fig. 16. — Neotizide. 40 mg/kg/day for 80 days. Severe microcavitation of cerebral white matter. H & E. Mag. x 450.

mono-amine oxidase inhibitors such as phenelzine and indanyl carbethoxy hydrazine. The histological changes are not unlike those induced in rats by the administration of triethyl tin compounds by Magee *et al.* (1957), in which

there is widespread oedema of myelin with spaces between the fibres but axons and myelin are intact. As with the hydrazine compounds, the exact pathogenesis of these lesions is unknown and it is interesting to see that there is a marked species difference in the reaction to this type of compound. Harper and Worden (1966) failed to show any cerebral lesion in rats with either isoniazid or Neotizide. Carlton and Kreutzberg (1966) showed the presence of similar lesions to those we have described in dogs in the brains of Pekin Ducks dosed with isoniazid; cerebellar ataxia and microscopical lesions were also seen in the cerebellum as well as in the optic lobe, spinal cord and cerebral hemispheres. The neuropathological picture produced by these compounds may be similar to a rare human condition of unknown aetiology reported by Buchanan and Davis (1965) known as spongy degeneration. It is also interesting to note the resistance of central axons to isoniazid toxicity as compared to the peripheral neuritis observed in some species. This fact further supports the hypothesis previously described that the axoplasm is more sensitive to noxious influences the further it is from the cell body (Cavanagh, 1964a).

CONCLUSIONS

The object of this paper has been to present a review of the toxic damage that can result when dogs are dosed with hydrazine MAOI and Isoniazid compounds. We have emphasised the correlation between the clinical symptomatology and neuropathological findings. It would appear from our experience that when neurological signs are present in a chronic toxicological experiment, morphological changes have, in all probability, occurred within the nervous system. It is, therefore, most essential that the utmost care be taken in the histological examination of the central and peripheral nervous systems. This point has been stressed most eloquently by Cavanagh (1964b), in which he also illustrated the importance of careful examination of neuromuscular junctions. The most satisfactory evaluation of neurotoxic drug effects are always obtained by clinicians specialising in this particular field (Palmer, 1965) with expert knowledge in neurohistological techniques and interpretations.

ACKNOWLEDGEMENTS

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APPENDIX II

ABNORMAL BEHAVIOUR IN THE DOG AND CAT

BY

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Reprinted from The Veterinary Record, Vol 71, No. 45.

Abnormal Behaviour in the Dog and Cat

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SUMMARY—Behaviour patterns in domestic dogs and cats are discussed with special reference to reproduction, the young animal, predation, eating and drinking, agonistic and social behaviour and senescence, and in appropriate circumstances comparisons are made with wild ancestors or counterparts.

Normal and abnormal behaviour are discussed in relation to experimental work involving dogs and cats and to the management and diseases of pet animals, with special reference to training, senility and the changes that may result from infectious disease and physical injury.

Introduction

THE Provisional Committee responsible for the selection of this paper preferred that its title should be restricted to abnormal behaviour, and indeed the original suggestion was that it should relate to neuroses and psychoses of these two species. Despite the undoubted existence of such conditions, from experimental as well as clinical causes, it is felt that they would at this stage scarcely provide adequate material for a paper, and that their understanding called for a discussion of normal as well as abnormal behaviour patterns. As in the human subject, the borderline between the two is not clearly defined, and it may be the situation and circumstances, some unusual or altered external stimulus, that causes apparently abnormal behaviour, not the innate or learned behaviour pattern itself.

The study of animal behaviour is developing rapidly, and I believe that it has much wider and economically more important implications in animal husbandry than studies on the neuropathics and psychopathies. The paper given by Wilkins (1957) to the Cambridge Congress, with its accompanying demonstrations, exemplified this, and outside the dog and cat field we have the problems of bull behaviour that confront the A.I. Centres.

There are available some extremely readable textbooks that present modern concepts, and I would particularly commend those of Tinbergen (1951), Thorpe (1956) and—in its relation to dogs and cats—the delightful semi-popular work of Lorenz (1954), to which numerous later references are made. Scott (1958) has much to say on dog behaviour, while Hediger's (1950 and 1955) works are of general interest to those concerned with domestic as well as with captive wild animals.

My own experience is limited largely to that of dog owner and to the use of experimental dogs for nutritional and toxicological research. On the clinical veterinary side, however, I have received considerable help from Miss Joan O. Joshua, to whom I am very greatly indebted.

Controversy over the phylogeny of the domestic dog

has been revived by behavioural considerations. Darwin (1868, pp. 15-43) reviewed the evidence then available for and against a polyphyletic origin of the different domesticated races and suggested that "We shall probably never be able to ascertain their origin with certainty". Since his day, however, there have been several studies that would seem to lend a strong support to an affinity with the true northern wolf (*Canis lupus* L.) rather than with the Asiatic jackal (*Canis aureus* L.) or with some unidentified common ancestor.

Miller (1920) suggested that, on morphological grounds, there was no evidence to support affinity with the jackal, and found that the teeth of a wide range of dog skulls were strictly of the wolf type. The combined cutting and crushing type of carnassials and molars were believed by him to be highly specialised, identical with those of the northern wolf, and far from the primitive form that one would expect in a polyphyletic animal. Miller considered that superficial resemblances in general form, in colour, and in quality of fur to jackals, coyotes, foxes and other Canidae were the basis for the erroneous hypothesis of a polyphyletic origin.

Wood Jones (1923, pp. 349-58) concluded that the Dingo, Warrigal or wild dog of Australia—to which the status of a sub-species had been given, *Canis familiaris dingo* (Blumenbach, 1780)—fell into line with all other races of domestic dogs in being of the true northern wolf type, and, moreover, that in respect of the large size of its last upper premolar, or carnassial, tooth it approached nearer to ancestral type than any other types of dogs that he had been able to examine. Following a discussion of the dental characteristics of dogs, wolves, jackals and foxes, Wood Jones wrote: "The loss of the cingulum on the first upper molar tooth is the hall-mark of the wolves—but it is the hall-mark of every breed of feral and domestic dog—and it is the hall-mark of the Dingo."

Iljin (1941), basing his conclusion on his study some years earlier of 101 wolf-dog hybrids at the Moscow Zoo Park, similarly supported a monophyletic origin of the domestic dog. He had shown that wolf-dog hybrids were fully fertile, and that in them the inheritance of eye colour, hair colour, hair pattern, ear form, body size and certain skull characters was identical with that in dogs. He could detect no essential differences in sexual behaviour, duration of pregnancy, age at which the eyes opened for the first time, eruption of the milk teeth, moulting, barking-howling behaviour (i.e., learning to bark in normal domesticated surroundings, but reversion to howling after a period of isolation), or even in what he termed nervous disposition. Usually, although not invariably, these wolf-dog hybrids were found to have only one breeding season in the year, like the wolf.

A popular review of much of this evidence has been provided by Vevers (1948). To add to it we have the work of Schenkel (1948) on emotional expression in wolves. His description, with illustrations (many of them reproduced by Thorpe, 1956), of facial attitude, of the colouring and bristling of the hair on the back, and of the use of the tail, indicate patterns of expression identical with those of domestic dogs. Indeed, the eleven forms of tail use for expression might have been portrayed direct from my own Husky, Bouncer, a veteran of the recent British Trans-Antarctic Expedition.

There have, however, been two studies, the results of which have been held by their authors to support the polyphyletic hypothesis, and each of them is based largely upon behavioural considerations. The first was that of Stockard and his collaborators (1941), who attempted to give greater definition to observed differences in the temperament of various breed crosses by use of conditioned reflex tests. They studied the parents and offspring of what were regarded as extreme behavioural types, viz., the lethargic typified by the Basset Hound and the active typified by the Alsatian or German Shepherd and the Saluki. Various blends, including extreme types of behaviour, were to be found among the cross-bred offspring, and in some instances the F_2 puppies resembled one pure breed in appearance but the other in behaviour. Stockard and his colleagues apparently believed that the many variations they produced when crossing achondroplastic animals with those without such skeletal defects implied a polyphyletic origin, but this view seems to have received little subsequent support. Their findings must be taken into consideration with those of Whitney (1932, 1948) on the inheritance of various "aptitudes", as he termed them, useful for hunting or other types of work, and with those of Scott and his colleagues (Scott, 1950, 1954, 1958; Scott and Charles, 1954; Scott and Marston, 1950; Scott and Fuller, 1951; Pawlowski and Scott, 1956; Fuller, 1953, 1955), to some of which reference is made later, as well as with various relevant findings summarised by Burns (1952).

A much more challenging view is that advocated by Lorenz, the second of whose two popular works was made available in English in 1954 and provides a clear description of his "jackal type" and "wolf type" dogs. Lorenz is of the opinion that there are a few breeds only descended mainly, although not entirely, from the northern wolf. These breeds include Eskimo dogs, Samoyeds, Russian Lajkas and Chow-chows. He regards almost all other breeds as being derived from the Asiatic jackal, and his book contains detailed descriptions of the behaviour patterns that are the basis of his contention. Lorenz is a recognised authority on animal behaviour, and we are indebted to him for his clear descriptive matter, some of which will be utilised later in this paper. Nevertheless, there would seem to be a need for much supportive information before his views on the jackal component could find general acceptance. Relevant studies might include those on jackal-dog hybrids analogous to those of Iljin with wolf-dog crosses, but with special reference to behavioural as well as morphological characters. Reciprocal jackal-dog crosses are possible, and

the hybrids are fertile in both sexes. Gray (1954), in her summary of the available evidence, states that the jackal $\times$ $\varnothing$ dog hybrids tend to resemble the jackal more than the dog in colour and conformation, but are timid and bite readily. Backcrosses to the dog are less timid and more good-natured than the F_1 hybrids. It is now fast becoming recognised that behavioural characters are of considerable importance to systematics. Nevertheless, this, like all new or relatively new applications, requires to be applied with circumspection to a given problem, and so far as possible to be taken into consideration with morphological findings. Pending further studies, it is likely that Lorenz's attractive hypothesis will not find acceptance with most authorities, notwithstanding the intrinsic value of his records of the behaviour of individual dogs and types. Scott (1958, p. 180), despite his own extensive work on dog behaviour, accepts the northern wolf origin on anatomical grounds.

From whatever wild canids the domestic dog may have been derived, it is highly probable that these were pack hunters and strictly monogamous. The fidelity of the dog fox and his excellent manners towards his vixen are models of male behaviour and constitute a problem for the fox breeder. They have been cited in relation to the old saw that two features only distinguish man from lower beasts: 'his capacity for drinking when not thirsty and his ability to make love at all seasons.' Unfortunately, or otherwise, domestication appears to have relaxed the dog's morals, and the dog breeder does not find himself faced with the same problems as the fox breeder in breaking the monogamous habit. Nevertheless, as Lorenz (1954) emphasises, male dogs behave with great chivalry towards bitches and puppies. He states that in the wolf this chivalrous self-control is extended only to females of the same pack, whereas in the breeds of dog he believes to be predominantly jackal in origin, it applies to every bitch, even if she is a complete stranger. "The Chow holds an intermediate position: if he always lives with his own kind he may behave loutishly towards strange jackal bitches, though I have never known one that actually bit them."

The relationship of mother to young is succinctly described by Thorpe (1956, pp. 396-7) as follows: "The response of the mammalian mother to her offspring and of the offspring to the mother are so intimately connected that it is difficult to consider them apart. The licking and eating of fluids and the after-birth by a mother seems often—in dogs, cats and rats—to be a necessary preliminary to the licking and grooming of the young . . . and is probably the basis of the learned attachment of the dam to the offspring; and if this is prevented there is evidence that the usual close link may not be formed. In wild ungulates the preparturient female segregates herself; but in domestic flocks of sheep the maternal drive, which is often at full strength before the birth, may be accidentally satisfied by contact with a newborn lamb nearby before her own lamb is born. The stranger may thus be adopted and the true mother vigorously repulsed by the foster-parent while her own lamb is actually being born. But though the individual is then recognised and adopted as a result of the learning pro-

cess, the instinctive phase is shown by the powerful 'desire' to adopt and nurse something. A virgin bitch will adopt almost any object of about the right size (e.g., a bone) and lavish all her maternal care upon it for a while (Russell, 1938). Similarly, the young mammal has a strong desire to find an object for the inborn movement—pattern of sucking, and if food is supplied without the normal amount of sucking, the sucking will appear as non-nutritional. This is well seen in the persistent 'over-flow' or displacement sucking of pail-fed calves, which will suck the ears or navels of their companions or—if kept alone—their own navels or anything in their pen that offers a mouth-hold. This 'displacement' sucking perhaps also accounts to some extent for thumb-sucking in infants. It has been investigated experimentally in puppies (Levy, 1934). Puppies fed from bottles having nipples with small holes sucked for an average of eighty minutes a day, and showed no displacement sucking, whereas puppies which had been fed through nipples with holes so large that their food requirements could be satisfied in thirteen minutes a day showed post-feeding displacement sucking which continued even during sleep. Confirmation from experiments in which puppies were deprived of sucking experience, although adequately fed, during the first ten days of life has been provided by Ross (1950). Similar evidence has been provided for kittens by Prechtl (1952).

Joshua (1959) has provided me with some extremely helpful information, based upon her own observations as veterinarian and dog-breeder, that is drawn upon freely in the paragraphs on puppy behaviour that follow. She has noted that intense respiratory stimulation, together with obvious crying, follows frictional sensations imparted by the bitch licking certain areas of the body, notably the muzzle and umbilical area. Use of this can be made in resuscitating fetuses delivered by Cæsarean section, in that gentle friction simulating that of the bitch's tongue applied to the muzzle and umbilical area of newly-delivered puppies will intensely stimulate respiratory effort. Similarly, friction applied to the perineal area or to the preputial area in males will stimulate urination and defæcation. Clinically, use can be made of this in orphan puppies in that they can be kept completely clean by applying gentle friction to these parts, so stimulating excretions with the possibility of cleaning them up and with no soiling of the bed between feeds.

The pattern of infant behaviour is that of sleep, feeding, play. In the first week or two of life sleep takes precedence and newly-born puppies and kittens will sleep probably for 90 per cent. of the time, waking only to feed and to be cleaned by the dam. Gradually sleep periods will become slightly less and feeding periods rather more prolonged, and, by about the third or fourth week of life, some slight evidence of a play pattern will be noted. Very shortly after the play pattern is noticed there is obvious sexual activity in that puppies at the age of three to four weeks will frequently make copulatory movements. This phase lasts only about two weeks and subsequently disappears until sexual maturity. The sucking instinct is very persistent indeed and, when puppies are three or four weeks old and the dam is leaving them for

longer periods at a time, during the waking part of the time puppies will frequently suck the preputial orifice of other males in the litter.

Observation of puppies in the nest makes it clear that excretory activity follows a certain pattern. From the age of about three to four weeks, when some locomotor activity is possible, it will be noticed that immediately on waking puppies attempt to leave the sleeping area and immediately urinate. They will then feed and, having fed, will be cleaned by the dam and will then indulge in a play period. Midway through the play period it will be noted that one puppy after another suddenly becomes pensive and will again urinate and probably defæcate. Considerable use can be made of this pattern of behaviour in the house training of young puppies. Nearly all books on the training of puppies recommend that, in order to instil habits of cleanliness, puppies should be put out immediately after feeding. This would seem to be absolutely wrong. A puppy should be put out immediately after waking, should be allowed to empty the bladder, should then be brought in to feed and should be carefully watched during the subsequent play period and, immediately there is a cessation of play activity, it should be transferred to the place intended for toilet purposes, when it will probably relieve itself again. Persistence of this popular fallacy that a puppy should be clean after feeding is not based on observation of normal behaviour.

Over the first few weeks of life, sleep periods will gradually decrease and be replaced by longer and longer periods of activity. It is essential that during the immediate post-weaning period long periods of sleep should be allowed. Far too many people, on obtaining a new puppy, make no allowance for the enormous infant requirement of sleep.

Scott (1958) refers to the habit in puppies, from about three weeks of age, of tasting various odorous substances combined with mouthing of pieces of wood or pebbles, as well as the bodies of other puppies, and states that stronger reactions are given to raw meat and to the vomit of the mother, both of which are eaten without hesitation if the pup is hungry.

Scott and his colleagues have divided the developmental period in puppies into four periods, which vary from one individual to another within relatively narrow limits: viz., a neo-natal period from birth until the eyes open; a transition period from then until the animal first appears to respond to sound, at about twenty days of age; a period of socialisation lasting until final weaning at seven to ten weeks of age; and a juvenile period from then until the animal is first capable of mating behaviour proper—as opposed to the juvenile activities already mentioned—at any time from six months onwards. Until the end of the transition period the puppy cannot be made to develop conditioned reflexes or to indicate other evidence of learned responses. Electroencephalograms first indicate a difference between sleeping and waking waves at about twenty days of age, and the adult form of electroencephalogram is attained between seven and eight weeks. Similar development appears to be the case in wolf puppies, which can readily be social-

ised to human beings if taken just before the eyes open, but which if taken a few weeks later have become extremely difficult to handle. The majority of dog puppies raised under kennel conditions with but little human contact to five weeks of age will show fear reactions which, however, will gradually disappear within the next two weeks if the pups are handled regularly. Puppies taken from the litter at three or four weeks of age, and raised by hand, do not show fear reactions at five weeks, but those allowed to run wild without handling to about twelve weeks of age become increasingly timid and may be extremely difficult to catch. Such puppies can be trained, but will always be more timid and less responsive than those socialised earlier.

There are important breed differences in the intensity of this timidity or man-reaction. Scott and his co-workers (Scott and Marton, 1950; Scott and Fuller, 1951; Fuller, 1953; Scott, 1958) have studied this factor and their findings include clear demonstration that the greater shyness at five weeks of a breed such as the Basenji, compared with a breed such as the Cocker Spaniel, is inherited on Mendelian lines, there probably being two genetic factors involved. Similar differences were found to exist in respect of crouching, which appears to represent the original setting that has survived in Cocker Spaniels since there has not been any selection against it. Modern setters are really pointers.

Timidity or shyness has been studied by many other workers. The wolf-dog crosses of Iljin (1941) have already been referred to, and these included some with an extreme passive defence reaction, while Krusinskii (1938) obtained similar animals from wolf-dog crosses and from crosses of German Shepherds (Alsations) with the Giliatski Laika breed. Thorne (1944) found that forty-three, or 73 per cent., of the F_1 and F_2 descendants of a shy, fear-biting bitch were shy, unfriendly animals, although the sires were normal friendly animals of other breeds, and that their behaviour, which appeared to be associated with a single dominant Mendelian factor, was not modifiable by training. As Burns (1952) states: "Such abnormally shy individuals also occur occasionally in pure-bred stock, and it is the general experience of breeders that excessive shyness is likely to be passed on to the progeny. This danger is particularly great in breeding from an excessively shy or nervous bitch, since, in addition to direct inheritance, the behaviour of the mother is likely to teach the pups to react as violently as she does to the presence of strangers or other disturbing influences."

Burns deals also with gun-shyness—to be distinguished from the "gun-nervousness" that is shown by dogs when first hearing shots but lost when the animals become accustomed to the gun—and cites the studies of the inheritance of grades of auditory sensitivity, by Humphrey and Warner (1934) in at least a partial explanation. Whip-shyness appears to be a similar phenomenon (Kelly, 1947). Humphrey and Warner found that stick-shyness was independent of gun-shyness, but that for both traits there were more under-sensitive males and over-sensitive bitches than would be expected if sex were not a contributory

factor. Kelly advises that the shy dog intended for use with sheep should be isolated early from all other dogs.

The study of agonistic, sexual and territorial behaviour, and of dominance relationships, is of the greatest importance in training, breeding, showing, experimental studies and veterinary attention. As Joshua (1959) points out, in puppies left together in groups a tendency to fighting may be noticed from a very early age. It occurs during the play periods and appears to be a response to unusual roughness on the part of a particular member of the litter, when there will be a slight flare up of tempers, a bit of a fight and then all will settle down. So far as is possible, this tendency should be checked at an early age, if it is intended to run dogs together subsequently. This really is the childhood period when the play and fighting patterns simulate those in the human child.

Real fighting comes later and is usually linked with territorial defence or sexual considerations. Tinbergen (1942) studied the territorial behaviour of Eskimo dogs and has since (1951) summarised his findings as follows: "The Eskimo dogs of East Greenland live in packs of five to ten individuals. The members of a pack defend their group territory against all other dogs. All dogs of an Eskimo settlement have an exact and detailed knowledge of the topography of the territories of other packs; they know where attacks from other packs must be feared. Immature dogs do not defend the territory. Moreover, they often roam through the whole settlement, very often trespassing on other territories, where they are promptly chased. In spite of these frequent attacks, during which they may be severely treated, they do not learn the territories' topography and for the observer their stupidity in this respect is amazing. While the young dogs are growing sexually mature, however, they begin to learn the other territories and within a week their trespassing adventures are over. In two male dogs, the first copulation, the first defence of territory and the first avoidance of strange territory all occurred within one week."

Joshua (1959) has noted in household dogs that there may be evidence of real male aggressive behaviour as early as four to five months of age, and before there is any sign of sexual activity at all. Fighting in the female usually starts at a later stage. At about six to seven months the first signs of sexual maturity will appear in that, in the male, the typical lifting of the leg will start, as also will some degree of interest of smells outlining his own territorial area.

During adolescence obvious signs of sexual activity are not uncommon. Young dogs will ride one another regardless of sex, they will ride inanimate objects and people's legs and this behaviour must be quelled at an early age if they are to become tolerable pets. Some dogs during this period will masturbate or will have so-called "wet-dreams", the latter being particularly common in toy breeds such as the Pekingese. It is a curious fact that at this age these animals, although apparently highly sexed, may display no interest in a member of the opposite sex if it is presented for mating. As a rule these signs disappear by the time the dog is fifteen to eighteen months old.

Dominance orders are clearly manifest among dogs within litters or colonies maintained together and in the animals of a neighbourhood. Wolves set up a definite dominance relationship as they devour their prey (Scott, 1958). Among the groups of dogs studied by Scott and his colleagues, two types of biologically maladaptive behaviour were observed, one involving sexual behaviour and the other fighting. Fuller (1953) records that: "Of course, mild or mock fighting occurs in all groups and does not result in any serious harm to the participants. The criterion for maladjustment, in the four cases discussed below, is the persecution by a litter of one or more members of the group, resulting in the death of the persecuted animal, or which, in the estimation of the experimenter, would have resulted in death if the victims had not been removed. In no case were any animals torn limb from limb, but some were excluded by their litter mates from shelter (in severe weather) and died as a result of exposure. In each of these four cases, the development of the maladaptive social structure is remarkably similar. Ganging up of a group (sometimes including the mother) on a single victim occurred first during the ninth or tenth week. In one instance, the first victim managed to become a persecutor, and the final victim was an original aggressor. In three litters, the initial victim remained a victim as long as it was a member of the group. In paired dominance tests involving control of a bone, the victim tended to be subordinate, but sometimes dominated one of the persecutors when they were matched as individuals. . . . The ganging up is specifically directed against individuals, and the persecuting group lives peacefully when the victim is gone. When we have returned a victim to its own litter after a vacation, however, the attacks soon start again. The victims . . . make reasonably satisfactory adjustments when placed with strange dogs in a friendly group and, although they have been observed to be aggressive towards their new associates, group retaliation does not occur. It is the combination of specific animals which produces the maladjustment."

In dog breeding and training, aggressiveness is usually regarded as of value only if limited. In the past, some breeds were, however, selected for their ability to fight, and Scott (1958) writes: "All of the terriers are fighting breeds. They are easily excited and relatively insensitive to pain, with tough skin on their necks and shoulders and strong jaws and teeth. On the other hand, many breeds have been selected for the opposite kind of behaviour. Hounds work in packs to find game, and fighting is highly undesirable. Beagles get along peacefully together, and even strange adult males can be put in the same kennel with little more than some growling and barking."

Humphrey and Warner (1934) found that for all types of training a moderate amount of aggressiveness was desirable, and that without it there was a lack of self-confidence and 'push'. With the decreasing aggressiveness that developed in the strain they were studying, there was a correlated reduction in initiative. These authors found that the quality of "distrust" was independent of shyness, being simply an avoidance of strangers and a refusal to make friends, unconnected with fear. This quality is of value in dogs

employed for army liaison work, but not in police dogs.

Lorenz (1954) has provided excellent and amusingly-illustrated accounts of encounters between dogs of different social dominance, and of one in which the two were of equally high ranking. The latter is a common sight, and from such meetings the two rivals will usually extricate themselves in a dignified manner if left well alone by attendant human beings—or bitches!

Smythe (1958) records the sad but interesting case in which a dominant hound turned upon one of its fellows, which repeated the behaviour with the next hound, and so on until the kennelman intervened with his whip. Almost immediately afterwards the pack fell upon and killed a Jack Russell terrier bitch owned by the kennelman that had hitherto regularly run with the hounds and had been on good terms with them. He records also the sudden destruction of other dogs, bitches as well as males, by individual Bull-terriers. Obviously, some releaser is responsible for this unhappy type of event, and Smythe points out that sheep-worrying may similarly occur spontaneously in animals that have hitherto led blameless lives, citing as an example a very gentle Labrador of his own. It seems certain that destruction of prey, however, is not actuated by feelings of hatred, and indeed is quite independent of fighting (see Lorenz, 1954). The sad cases of man-killing by dogs have been investigated by Grzimek (1954a, b), while Lorenz emphasises the importance of the fence, cage-bars or similar barrier in dealings not only with dogs but also with wild animals. He points out that noisy and aggressive behaviour on the part of a dog behind the fence usually subsides rapidly, to be replaced by flight, when one opens the gate, but that conversely the shy dog or wild animal may attack once the flight distance has been transgressed by stepping into the garden, room or cage. Attack may usually be thwarted by standing one's ground, and I have vivid recollections of the behaviour of a Bull-mastiff that rushed towards me as I entered by foot the drive of its home, literally skidded past me with "brakes full on" as I stood stock still, growled menacingly while I refused to budge, and suddenly accepted me to the point of accompanying me to the house. Similar experience must be common in veterinary practice.

Lorenz provides also a clear explanation of the relationship of man to the dog pack and his acceptance as a leader. He suggests that failure of the adult male wolf to accept this situation is a basal reason why it, but not the wolf bitch, is usually impossible to train as a pet, even though trained from an early age. In our own laboratory we had an interesting example of the changes that may occur with age in a Husky pup, Joey, presented to us by a member of the British North-Greenland Expedition. When young, this pup was clearly dominated by a Scottish Terrier puppy, Jack, with whom he was brought up. As he grew larger he lost no time in asserting his authority, and every morning, when the animals were let out of their runs, he would take a token grip of the back of Jack's neck to indicate his superiority. One morning, in error, he did the same to a mature Scottish Terrier, Scottie,

which reacted in no uncertain fashion to this insult. Whereupon Joey lay supine, with his neck exposed in complete surrender. Later Joey shared quarters with two Golden Retrievers, Peter and Paul, and at first made almost exaggerated efforts to indicate his subservience to them, but now that they are ageing and he is mature he attempts, not always successfully, to put them in their places. I would agree entirely with Lorenz that the most effective means of punishment is to shake the dog by the scruff of the neck. "Since dogs, like monkeys, do not hit, but bite each other on their ranking order disputes, the blow is not really an adequate or intelligible form of chastisement . . . one can imitate the penal methods of the pack-leader and involve one's own personal feelings much less by lifting the dog up by the neck and shaking him. This is the severest way I know of punishing a dog, and it never fails to make a deep impression on the offender. In actual fact, a wolf-leader which could lift up a dog of Alsatian size and shake it would be a giant, a super-wolf, and, as such, the dog regards his master in the moment of chastisement. Although this form of punishment seems to us much less severe than beating with cane or whip, we must be very chary of using it, even in adult dogs, if we do not wish to intimidate them altogether."

The marking out of territory by urine is an inborn habit that has to be suppressed for satisfactory domestic relationships, but will be revived at once if that territory is invaded by a strange dog. Lorenz considers it unfair to take one's dog to a household where another dog is kept, if only in regard to the housewife's feelings at the effects upon her soft furnishings. Smythe (1958) describes, in relation to another form of releaser, unwanted urination in the house—coupled with other behaviour regarded as unwelcome: "So nowadays, when your musical sister bursts into song and Carlo, the Alsatian, lifts his head and his voice and joins in the chorus, remember that he is merely doing what his instinct warns him is his duty, and by so doing he is enabling the rest of the pack, now comfortably stretched out in their arm-chairs, to slumber in complete safety."

"Should some untutored pack-member, even the pack-boss, turn upon Carlo and scold him severely, the poor dog will experience embarrassment and 'conflict', a battle between what he knows he ought to do and the rebuke he has received for doing it. In order to cover up his feeling of distress, as every good pack-dog must, he will probably do something comparable with what a human being might do in similar circumstances, although the exact details of the behaviour may not be identical in the two cases. Carlo will employ what is known as a 'social-releaser' . . . (and) . . . will sit down, scratch his ribs violently with one hind foot, run around the room and possibly embarrass the whole company by cocking his hind leg against that of the table. If he is again scolded for this natural response, his nervous mechanism may go haywire, and it really is not to be wondered at that so many thoroughly respectable dogs compelled to live with very dense humans so often develop hysteria or have to be treated for 'fits'."

Urine may be voided as part of a submissive

response, e.g., when a puppy is scolded or encounters a very large dog and, as Lorenz describes, as part of the process of disengagement between rivals of equal social rank. Many owners fail, of course, to recognise the submissive response of the puppy, and associate urination with excitement or scold their new pets without realising that they are receiving tokens of deference. These behavioural as well as excretory functions constitute a complication in studies on dogs housed in metabolism cages. In small cages, many dogs will not mark out their territory initially, but will hold urine often for well over twenty-four hours (Worden, 1939). The same inhibition may occur in veterinary hospitals or infirmaries, especially when the new patient is confined to small quarters. With a large metabolism cage that permits of free walking about, the difficulties are greatly lessened (Worden and Waterhouse, 1956).

Within the age range corresponding to Scott's period of socialisation, the vast majority of puppies will in appropriate circumstances begin to form a strong attachment to an individual human being. This may persist or wane according to type—Lorenz describes clear differences between his wolf and jackal dogs in the pattern of the future relationships with man—but there is often a phase of adolescent independence when the dog will become disobedient and defiant. Discipline must be persisted with at this period, but patience also shown, and the animal will normally become more obedient again at the age of eighteen months to two years (Joshua, 1959).

Krusinskii (1946) is reported to have studied the correlation between constitution and behaviour, basing his data on 241 dogs (German Shepherd and Eskimo breeds and dogs of no specific breed) classified according to their constitutional type (eurysonic, leptosomic, "svelte" or cerebral, athletic). There was only a slight relation between degree of excitability and constitution. Leptosomic and cerebral dogs were more excitable than those with eurysonic and athletic constitutions. There was slight relation between training ability and constitution. There was slight positive correlation between active defence reaction and athletic constitution, but not between active defence and eurysony or leptosomy. There was no correlation between constitution and passive defence.

Vaux (1953) found differences between the Dackel, Chow, Pointer and Poodle in their behaviour towards prey, and considered that some of the behavioural changes occurring during domestication had become genetically fixed breed characteristics.

The drinking pattern of the dog is of clinical and experimental interest. Tarbin (1955) has shown that dogs consume larger volumes of water daily at higher environmental temperatures by taking more frequent drinks rather than by any change in the amount of water taken at each drink. Indeed, a given dog appears to favour a characteristic size of drink that is not markedly affected by total food or water intake. It is nevertheless a fact that adult dogs with kidney damage, presumably of chronic interstitial type, will consume very long single drinks.

Dogs are equipped with remarkably good senses of hearing and smell. Engelmann (1928) found that their

sound localisation was about twice as accurate as that of man, while Kalmus (1955) has shown that the capacity of the dog to detect the individual odour of human beings is quite remarkable. This individual odour is perceived by the dog, even when mixed with another person's odour, or with some strongly smelling substances. In tracking experiments dogs were found to be able to distinguish even between the odours of identical human twin sisters.

Sight is of less importance to some canine activities than others. Vevers (1948) states that the Greyhound, Whippet, Borzoi, Saluki, Afghan, Irish Wolfhound, Scottish Deerhound, Otterhound, Elkhound and Dachshund all hunt by sight, and that the smallness of the optical angle suggests that the dog has good binocular and stereoscopic vision. Dogs are able to discriminate between differences, of size that are considerable, but are less certain with smaller differences, while their discrimination of form is poorly developed, despite the claim of some observers to have trained dogs to distinguish between squares, circles, triangles and other forms (see Warden, Jenkins and Warner, 1936).

Senile changes in dogs are very interesting and appear to follow a fairly definite pattern, not necessarily related to physical deterioration (Joshua, 1959). Hearing fails frequently in old dogs and the first sign that it is failing may be the animal's inability to detect the direction from which a sound is coming, although he can quite clearly hear the sound. This may lead gradually to total deafness, or total deafness may ensue quite suddenly. Such animals can be taught to respond to signs, but, of course, they become an increased responsibility as they have to be watched very much more carefully in relation to traffic and other hazards. Sight on the whole remains good, as does sense of smell.

Senile temperament changes become obvious in that the dog's conscience becomes slightly less active in respect of certain adult habits, particularly cleanliness. For example, dogs which have flatly refused to defæcate other than on certain specialised areas such as grass, may begin using roads or even pavements without any obvious anxiety, and in some animals even house cleanliness is lost as time goes on. These animals also, usually, become much less anxious about external stimuli; for example, dogs which have been very nervous of thunderstorms, bangs and so on become completely unperturbed as age advances. This may be associated with failing hearing, but not necessarily so.

Personality or mental changes may follow diseases which affect the nervous system; for example, hard pad encephalitis. Certain dogs, if they acquire the disease in adult life, are left with a markedly impaired intelligence. Some puppies which get a mild encephalitis during fairly early life may be left mentally retarded. Occasionally, a previously tractable, good-tempered dog will become unreliable. Similarly, temperament changes may follow accidents and, in the case of old dogs, an accident of shock which does not cause any serious physical damage may result in a rapid degeneration of mental ability. MacCurdy (1944) recorded in detail the changes in a male Dachshund,

aged 4½ years, following concussion. Immediately afterwards there was a period of acute confusion and after this two discriminable types of mental abnormality. One was a general dementia not unlike senile decay in man, with loss of sexual and social drives, reduction of hunting tendencies and a general, rather seclusive, lethargy. The other was more like a "functional" human deterioration. There was regression not merely to puppy habits, but also what looked like wild canine behaviour. An interesting phenomenon was the apparent wiping out of a neurosis by the dementia, although its belated reappearance was the indirect cause of the dog's death. MacCurdy likened this to a weakening of complexes in man with the development of arteriosclerotic or senile dementia.

Jealousy and possessiveness are often displayed by dogs. Gun dogs, in particular, are extremely possessive, and may create great problems over possessions such as rubber bones and so on, when they may hold an entire family at bay. Jealousy is displayed very often if affection or notice is paid to any other animal.

Among cases of aberrant behaviour is one observed by Joshua (1959), viz., a young male adult Alsatian, a dog apparently extremely well-balanced, of excellent temperament and absolutely normal everywhere except in the back garden of the owner's house. When let out into the back garden, it behaved normally for about two minutes, would then start to look round in a thoughtful and apprehensive manner, and at that point would start chasing its tail. This it would grasp at the base, and would then chase it persistently until it fell exhausted. No reason for this behaviour could be found; it seemed unlikely that it was anything to do with the electric railway running at the bottom of the garden, but in every other place the animal's behaviour was absolutely normal.

Comben (1955) has recorded a similar case, in an otherwise healthy Airedale-type crossbred about two years old. "When he came into a room he would behave normally for a few moments, then suddenly stand rigidly with his four feet well spread out and stare fixedly at a point on the carpet as if in a trance, with not the slightest movement for one, two or up to five minutes. Then he would spring up in the air and prance upon this point, several times running. Thereafter, with hardly a moment's respite, he would move off excitedly and start the whole process again on some other point on the carpet. He would select some five or six spots all told and the whole performance would last anything up to twenty minutes."

This behaviour is said to have ended when the dog was allowed to go out on his own. "Within a week he had been seen consorting with bitches on three occasions. After ten days his strange habits in the house showed signs of abating, and after three weeks he was completely cured."

Joshua (1959) states that claustrophobia, in the Bull-terrier particularly, is not uncommon, and such dogs, if shut up in a kennel, will indulge in wild tail-chasing until they fall exhausted, or show some other similar evidence of their phobia at being shut up. It is even impossible to keep some dogs in a normal-sized room with doors and windows shut.

Another form of abnormal behaviour may be seen

in the exaggerated response of the bitch to pseudo-pregnancy, with nest-building (even to the extent of removing wallpaper or other suitable material) and even lactation. Bloom (1954) is careful to emphasise the range of circumstances in which pyometra may develop, but Smith and Jones (1957) state what must, I believe, be widely held among practising veterinarians, viz., that in the bitch, as in the female cat, many cases follow pseudopregnancy.

In view of the way in which stud matings are conducted, it is not surprising that there are many behavioural difficulties among both dogs and bitches. The full development of mating patterns call for appropriate surroundings and build up, and in species in which there is naturally a strong pair-bond the highly artificial nature of the stud is a considerable obstacle. Graham (1954) records that only a relatively small proportion of stud dogs form what he calls the "cave-man type", able and willing without delay to execute service with almost any bitch presented to them. All veterinarians connected with dog-breeding know the difficulties encountered in attempting to get some bitches to mate, a procedure that may call for forceful restraint. Goldstein (1957) records that there need not be such a specific act as biting the male by the bitch to keep him off, and that an individual bitch—among a colony of experimental dogs—may consistently fight off one male but readily accept the mating advances of another. Joshua (1959) mentions an interesting case of a bitch that resisted all attempts by stud dogs, but accepted a litter brother immediately. It is scarcely surprising that in some instances a pedigree bitch may fail to accept a stud dog, but present her owner with a healthy litter from what is usually termed mesalliance with a local mongrel of her choice.

Male response to a bitch in oestrus may be masked. Adie (1953) records the successful prevention of unwanted matings in a group of Huskies by the use of a proprietary preparation based on essential oils, and I have witnessed the complete deception of one of our own dogs—a crossbred terrier, Kim, whose usual reaction to bitches would place him in Graham's cave-man class—when actively courted by a bitch suitably treated with chlorophyllin. He was pleased and flattered by her attentions, but quite failed to associate these with mating drive.

The response of the male dog to castration shows considerable individual variation, as the studies of Goldstein (1957) on Beagles have shown quantitatively. Some show a consistent decline in mating behaviour, comparable with that typical of rodents. Others, after an initial period of decline, showed a drive equal to, and even greater than, that before castration. The effect of castration was usually to lower the ability to lock with the bitches, i.e., the period of intromission was reduced and was presumably not accompanied by the changes necessary for locking to occur. This could be reversed by the administration of androgens.

Goldstein found that surgical removal of the pyri-form cortex of male Beagles did not effect a general improvement in sex behaviour, as described for cats by Schreiner and Kling (1953), but that there was a marked increase in the speed at which these dogs mounted the bitches at the start of each test. It may

seem strange to discuss dog behaviour without specific reference to the work of Pavlov (whose synthesis was made available in English in 1927) and to all the work that has followed from him, but I want merely to mention the present-day distinction between the classical conditioned reflex of the Pavlov school and trial-and-error learning (see Konorski, 1948; Thorpe, 1956).

Belief in the popularly termed "sixth sense" of the dog is widespread, and one is always coming across almost incredible stories—told usually in an attempt to demonstrate the dog's "intelligence". It must be conceded that we have not as yet adequate explanations of homing behaviour or of many other surprising canine feats. I can add one bona-fide case, in a Scottish terrier, Sheila, who was devoted to me and who remained with my parents at East Barnet from 1940 onwards, when she was seven years old, while I was working at Cambridge. As often as I could—but sometimes at intervals of some weeks—I would return home, or call in on my way to London, but my visits were usually unannounced, at different times of the day, and by any of a considerable number of local trains with which I had connected at Hatfield. Yet almost invariably Sheila would rise in pleasurable anticipation when the particular train bearing me was heard, some half a mile away, approaching the local station, and thereby inform my mother of my coming. There were over thirty stopping trains a day travelling in that direction.

The Cat

The ancestry of the domestic cat, *Felis catus* L., is also somewhat in doubt, and Pocock (1951) considered it probable that it is a composite form of which the European representatives, at all events, owe their origin to the crossing of the two wild species *Felis silvestris*, Schreber—the sub-species of which, *Felis silvestris grampia*, Miller, is the wild cat of Scotland—and *Felis lybica*, Forster. Some authorities have attempted to place these two species with *F. catus*, for they are obviously closely akin and have not any constant difference of skulls or teeth. Pocock, however, did not adopt this view, since he could not find any evidence that *F. silvestris* and *F. lybica* integrate in a wild state. He considered it probable—with due qualification—that domestic cats with *torquata* or "striped tabby" markings were hybrids between *F. silvestris* and *F. lybica*, while those with *catus* or "blotched tabby" markings were mutants of the other, there not being any recorded examples of the *catus* pattern in wild examples of either species. It is interesting that Pocock should have written: "There is indeed less to be said in favour of applying the name *catus* as a specific term to these two kinds of wild cats than for calling the wolf *Canis familiaris*." Darwin (1868, p. 45) wrote pertinently: "Whether domestic cats have descended from several distinct species, or have only been modified by occasional crosses, their fertility, as far as is known, is unimpaired." Hamilton (1896) reviewed evidence of crosses between domestic and wild cats and Peters (1932) reported on hybrids between *F. catus* and *F. silvestris* that resembled the wild parent and were heavier for their age than *F.*

catus. They were distinctly wild, spitting on handling almost before they were able to crawl. I have noted the habit, as doubtless many others must have done, in lion cubs only a few days after the eyes are opened. Pitt (1944) doubts the existence of *F. silvestris* blood in *F. catus*, but writes: "These two cats will mate and their offspring are fertile, but the hybrids show almost complete dominance of the wild-cat type. They are rusty-hued striped tabbies with thick tails ringed with black, which tails terminate more abruptly than the domestic cat; and they inherit the sporting disposition of the wild cat, besides being nervous and queer-tempered. My crossbreds could not be trusted free after they were half-grown, because they attacked poultry and ducks. Had I not shut them up, they would have been off to the woods." She states also: "The wild cat has . . . an undoubted tendency to monogamy, trait at marked variance with the promiscuous behaviour of our household puss."

Lorenz (1954) has provided beautiful descriptions of the play of kittens—activities which, as Thorpe (1956) states, provide evidence for the existence of inborn hunting behaviour. Kittens and other cats are, of course, attracted by almost any moving object. Joshua (1959) points out that since the majority of cats are neutered at an early age, and entire animals indulge in sex behaviour out of doors, the development of sexual signs and therefore of certain forms of adolescent activity are either absent or unseen. Occasionally, however, neutered males either behave like oestrous females or will ride objects in the same way as entire puppies. So far as can be ascertained, such behaviour is not associated with remnants of gonad tissue.

Leyhausen (1956) and Baron, Stewart and Warren (1957) have recently made substantial contributions to the study of social and other behaviour patterns in cats and their papers should be consulted for references to earlier studies. Leyhausen describes activities directed towards the catching, killing and eating of prey—for the release of which there are at least seven sign stimuli—and points out that the first of these are frequently directed towards substitute objects, or may even "go off *in vacuo*". Whether or not a domestic cat will kill rats is dependent upon a number of factors, mainly its "courage", which, in turn, is correlated with social rank order. Very few cats fail to be intimidated by the counter-attack of a cornered adult brown rat, and as a predator the domestic cat is specialised on much smaller rodents. Notwithstanding these findings of Leyhausen, cats may be used effectively in farm rat control (Elton, 1953). Kuo (1930) had earlier studied the development and variability of the rat-killing habit in cats, and had recorded that of twenty kittens reared alone, only nine killed rats or mice without any training, whereas eighteen out of twenty-one kittens brought up in the environment where killings were going on killed rats or mice before they were four months old. Hunger was not a factor, and cats conditioned to a vegetarian diet would kill rats, but not eat them. Leyhausen, however, considers that the whole sequence of experience from stalking to killing matures irrespective of individual experience, but that some reinforcement is usually required for the killing

bite to gain its full strength. Lorenz points out the clear distinction between kitten play directed towards predation and that which stimulates fighting: "Instead of leaping on its brother as on prey—an action which may alternatively, of course, be performed—it assumes a threatening position while still galloping on, arching its back and advancing broadsides on." The fight of adult toms is described—as Lorenz says in all its complicated ceremonial—by Thompson, and Leyhausen similarly provides a graphic account. The aim—once threats have failed and actual combat begins—is always to aim at biting the nape of the adversary's neck, and so bring him down in the same way as tall prey.

Joshua (1959) finds, however, that injuries to males during fighting appear to be inflicted on repeated occasions on the same site, and that this site may be variously placed on the body, this being by no means always on the nape or head. It would seem as if fighting patterns may differ in different individuals.

Cats will defend their homes against neighbouring and intruding cats, and also possess a fairly large hunting territory which they will guard against trespassers. There may be partial overlap between neighbouring territories. An encounter between stranger cats is enacted according to fixed rules. The territory owner usually has the better of it, and as the superior cat attempts to smell the nose and anus of the intruder, it attempts to proceed with the exploration of the strange territory. Even quite young kittens will attempt a thorough exploration of a new room (Russell, 1938).

Leyhausen states that a true submissive attitude does not occur in adult cats, which as solitary animals do not establish rigid social orders if kept by numbers in a fairly large room. "One or two despots may emerge but enjoy very limited prerogatives only. One or two animals may become 'pariahs'. But neither despots nor pariahs develop of necessity, and, anyway, the rest of the crowd is on equal footing, so that the outcome of encounters cannot be predicted from the respective ranks of the animals involved. In the social intercourse of cats crowded in confinement, full intensity performances of either attack or defence behaviour are extremely rare. The single motor patterns of these types of behaviour gain a peculiar independence and appear to be at the disposal of purposive behaviour. They do not then any more express the cat's actual 'mood', but are used according to the social effect which the individual 'wants' to achieve. Low-ranking animals regularly are forced to be much more demonstrative than high-ranking ones in order to attain the same social results." The cats in our experimental colony at Huntingdon are maintained in outdoor cages, with small heated sleeping quarters, and with shelves and branches of trees to provide adequate opportunities for activities off the ground. For the most part, males and females are maintained in separate groups, and it would seem that one of their principal "wants" is to indulge in homosexual behaviour, possibly as a substitute for normal sexual activity. We have never had the slightest difficulty in breeding in captivity—quite the reverse—and Scott (1957) reports similarly on her colony at the Royal Free Hospital School of Medicine. It would seem

that the many reports of failure to breed experimental cats are due to failure to provide adequate nutrition, a suitable background for mating behaviour patterns, a control of infectious disease—sometimes, perhaps, to all three. I hope that at the end of this paper it may be possible to show a short film, for the loan of which I am indebted to Professor E. C. Amoroso, on mating behaviour in the cat. As Leyhausen states, in the course of the mating period the male is the first to take the initiative, and later it is preponderantly the female, while the partners of a pair may meet again in a long series of matings. As will be seen in the film, the female has a most pronounced “after reaction” to coitus and will violently reject any attempt by the male to repeat it too soon.

Leyhausen considers that while in more primitive carnivora, such as some Viverridae or Mustelidae, the male grips the female by the neck before mounting in order to block escape reactions, this has tended in the case of the domestic cat to become a ritual. Although the male may try to hold on to a female and to mount her by force, he will not be successful unless she gives in voluntarily. “In the Pantherinae, the copulatory bite has been reduced to an accessory expression of orgasm.”

Joshua (1959) points out that acquiescence, including the necessary postural changes, on the part of the female would seem to be essential for mating to occur.

Comfort (1956) made a study of longevity of cats, including six entire males of over 19 and in one case 27 years old. Mating continued throughout life in some of the oldest males investigated.

Parental care is highly developed, and, as Lorenz states, defence of the young is the one contingency in which a cat may make a prolonged and earnest attack while in the hunch-backed attitude with which it normally keeps a dog or other enemy at bay—and from which it will otherwise attack only if the “critical distance” is overstepped by its adversary. Retrieving of the young is dependent upon what many behaviourists term an “innate releasing mechanism” responding to the wail of a lost kitten.

The findings of Baron *et al.* generally confirm the asocial and individualistic behaviour of cats in relation to their fellows. They noted dominance hierarchies, but found that among cats living communally these were susceptible to change. They reported that aggression does not seem to have any direct relation to success in food getting, nor is it necessarily a concomitant of downward displacement in the dominance order, or of prolonged frustration in food getting. They consider that dominance status in the cat is not a consistent function of sex, weight, passivity or success in learning problems.

The reaction of cats to dogs and to man has received considerable study. Most people look upon cats and dogs as sworn enemies, and Spurway (1953) has produced a somewhat surprising hypothesis to account for their relationship. Nevertheless, the kitten does not seem to have any inborn understanding of the facial expressions of the dog. As Lorenz (1954) writes: “I am perfectly certain that, without previous experience, cats do not understand the expressive movements of

dogs, although they are so similar to their own. Cats which are on friendly terms with dogs in the same house display a trustfulness towards strange dogs which may, and sometimes does, lead to their own ruin. I have often observed how such a cat will stare with fearless innocence full into the eyes of a strange dog which is bracing itself unequivocally for attack. It is equally unusual for a cat-friendly dog to understand the threatening of an angry cat, unless it has already learnt to do so by bitter experience. This is quite remarkable, for one would surely expect the growling of the cat to be intelligible to the dog, which expresses its emotion in just the same way.” Lorenz again is responsible for a detailed account of play between a dog and cat, but reports one case only of what he regards as a true bond between members of these species, built up apparently on the basis of something akin to maternal feelings on the part of the female cat for a somewhat weak and cowardly male dog which she accompanied on walks and even defended—successfully—from attack by a strange dog.

Many cat owners are proud of the independence of their charges and Lorenz emphasises the dual role of the cat that hunts and fights out of doors but becomes a peaceful pet within. He states that, in spite of their well-defined, wild, private lives which often kept them from home for days on end, his most temperamental and virile cats were, at the same time, the most affectionate. He utters a stern warning to those who are sufficiently respected by cats for the latter to accompany them on walks: “A fully grown cat in good health and strength is unable to follow the leisurely pace of a strolling man even for half an hour without showing signs of exhaustion.”

Thorpe (1956) suggests that the cat, alone among animals below the primates may possibly be capable of true imitative behaviour. He believes that most forms of apparent imitation are due to local enhancement, in which the animal's attention to a particular object or to a particular part of the environment, is drawn, and that habits acquired by trial-and-error learning on the part of pioneers (e.g., the tits that have learned to pierce the covers of milk bottles) will spread in this way. True imitation he defines as the copying of a novel or otherwise improbable act or utterance, or some act for which there is clearly no instinctive tendency. Whether or not true imitation according to this definition, the aptitude of some cats for copying is quite remarkable. I once owned a male cat, Ju-Ju, that quite spontaneously copied one of my Scottish terriers, Sandy, in begging at table, and I am quite sure that such examples are frequent. As a boy I succeeded in training young kittens, still with their mother, to stand up and box with my fingers and I well recollect going out with my father one day to find two of them indulging in a boxing match of their own. With patience, young cats will learn to play simplified forms of hide-and-seek, and it seems likely to me that in many respects their learning of tricks may differ considerably from those of the large cats familiar in the circus, training for which consists basically of the utilisation of natural and often inborn abilities (see Hediger, 1955).

Cats served extensively for the studies on learning of Thorndike, inventor of the "puzzle box" method, and of the others who followed him and severely criticised his findings, including Adams, whose string-pulling techniques helped to demonstrate the part played in insight learning by conation and perception (for references and discussion, see Russell, 1938).

Experimental surgical procedures have done a great deal to localise the centres responsible for many facets of cat behaviour (for references see Tinbergen, 1951; Goldstein, 1957; Scott, 1958). Although not strictly relevant to the present paper, the findings are of intrinsic interest and would suggest that observations on behaviour could well help to identify the site of lesions arising from accident or neurotropic infections. Many elements of the female mating pattern—raising of the pelvis, treading and contralateral swinging of the tail—may be induced by stimulation of the perinaeum in animals with the spinal cord cut at a high caudal level and kept alive by artificial respiration. It has been reported that the same response will occur in anoestrous females and even in males so treated. Male cats from which the three sacral spinal segments have been removed show aggressive sexual behaviour despite paralysis of the hind limbs, absence of sensitivity in the genital region and, of course, failure to gain intromission. Unilateral removal of the cerebral cortex does not inhibit copulatory behaviour in the male, but bilateral removal does so. Frontal lobe lesions appear to affect the motor abilities, but not the sexual excitability of males, which will follow and call to females and attempt a large number of mounts, although able to gain intromission in only a small proportion of these. Bilateral lesions of the temporal, parietal or occipital lobes has little effect upon mating, although with considerable invasion of the occipital areas there is difficulty in finding the female. In the inferior temporal lobes, bilateral injury to the amygdaloid nucleus enhances the sexual behaviour of the male, which will show a greatly increased tendency to mount females, or, indeed, other available animals such as small rabbits, dogs and chickens. This hypersexuality, as it has been called by Schreiner and Kling, can be reversed by castration but reintroduced by injections of testosterone propionate—an interesting demonstration of the interaction between neural and hormonal effects. These hypersexual males are benign, having become easier to handle and difficult to rouse to anger. Females with similar lesions likewise show signs of hypersexuality, but instead of becoming docile are inclined to a greater irritability.

Cats that have had bilateral removal of the cerebral cortex have to be fed by hand, since they cease to attempt to eat voluntarily. When picked up they tend to fly into a rage and their tendency to fight is increased.

The work of Hess and his colleagues, who have systematically probed the hypothalamic region by the introduction of minute electrodes, has localised areas where the application of a stimulus will elicit the complete behaviour patterns of fighting, eating or sleeping. All the elements of each pattern are displayed in perfect co-ordination, and the response is initiated by genuine appetitive behaviour, for the cat will seek

combat, a sleeping place or food according to the area stimulated.

The early castration of male cats precludes sexual experience. In older animals the effects of castration upon sexual behaviour depend upon previous experience, those with a previous experience of mating taking much longer for their activities to decline (Rosenblatt and Aronson, 1958).

Cats, and particularly Siamese, will display resentment, very often, for example, at the introduction of another kitten or a puppy, and a common sign of such resentment is spraying, even in a neutered cat. Many neutered males will go round spraying when there has been some change which has aroused feelings of jealousy (Joshua, 1959). An excellent example of this occurred in a neutered male which had for several years been the favoured pet of a member of my staff, and which reacted in this way to the arrival of her baby.

Cats appear to suffer from shock in the nervous sense rather more readily than do dogs, and it was noteworthy during the war years that many cases of apparent shell shock in cats were encountered, whereas in the dog this was rare. Some cats became so shocked that they could not be got out from darkened corners and had to be destroyed on humanitarian grounds. There is also on record the so-called fly-catching syndrome which resulted in some cats after the severe East coast flooding (Joshua, 1959).

Cats are very subject indeed to so-called mental trauma and cases of undoubted nervous anorexia do occur. One case brought to the notice of Joshua was that of a blue Persian cat which had been given relief for rectal impaction by enemata and manipulative treatment, without general anaesthesia being induced. Although the cat made a perfectly satisfactory physical recovery, it refused to eat for some two months afterwards, became very dull and stuporous, and it was not until the cat was treated with tranquillisers, sedatives and vitamin pills that it was restored to normal. Many timid, non-aggressive cats will suffer severe mental shock after being involved in a fight with another cat, or more particularly if attacked by a dog. Although there is no physical injury, such animals frequently require treatment of the nervous system before they are restored to normal.

Lorenz states that cats always announce their intention of attacking and—"except in the case of unreliable or mentally deficient psychopaths"—never bite or scratch without giving previous unmistakable warning to the offender: usually, in fact, the gradually increasing threatening gestures are suddenly exaggerated just before action is taken.

Discussion

It has been possible to discuss a selection only of the available material, and there is a great deal of experimental work dealing with normal and abnormal behaviour—and also many clinical communications—that have been omitted from consideration or inadvertently overlooked. Nevertheless, an attempt has been made to indicate the interest and value of behaviour studies in relation not only to abnormal cases in the narrow sense but also to a better understanding

of the dog or cat as a pet, working animal, experimental subject or patient. It is to be hoped that the growing reciprocity between veterinary science and subjects such as experimental physiology will be paralleled in a similar relationship of the former to behaviour and comparative psychology. It was pleasing to read in the recent reported production of experimental atherosclerosis in dogs (Marmorston, Rosenfeld and Mehl, 1959) that one of the physiologists concerned was also a veterinarian who could readily distinguish the accompanying neurological changes from spontaneous conditions.

It is tempting to indulge in comparative considerations, and one wonders to what extent the study of aberrations, such as that shown by Smythe's Labrador, may be of help to criminologists, but we may content ourselves by quoting from Thorpe (1956) to the effect that, since Darwin's day, comparative psychology has shown "... the extraordinary superficial similarity between much animal and much human behaviour. This is true whether we speak of drives or orientation, of trial-and-error, of insight or of imprinting. Even such characteristically human behaviour as the framing of language and of the concept of number, the use of symbols and artistic appreciation and creation, seem no longer to offer quite the clear-cut differentiation between man and animals which was formerly supposed. And if and in so far as it proves true that the concept of purpose is necessary in the description of animal behaviour, another important similarity will have been established. In any event, it seems that ethology is already confirming the conclusions of other biological disciplines, and in doing this it may in due course provide many facts which will assist human beings in the better ordering of their social relations."

In the domestic animal field we are not faced with mass public objection to capital punishment, and while cases of the type mentioned by Smythe should surely go free, there is no justification for retention of the confirmed killer dog—be it of other dogs, cats, sheep or other animals.

I have expressed my thanks to Miss Joshua and to Professor Amoroso, and I should like to thank also Mr. Jack Pickup of Barnet, who, in the limited time available, taught me a great deal. Finally, I think that the late Dr. J. T. Edwards would have been delighted to know that this subject was to have been discussed today.

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THE SPEAKER'S INTRODUCTION

The Chairman, Mr. J. Wright, introduced the author and invited him to present his paper. Mr. A. N. Worden, in presenting his paper, said that in accepting the kind invitation of the Provincial Committee to present the paper, he had been concerned on account of its lack of original material. Despite the fact that he had worked with dogs and cats over many years, their normal and abnormal behaviour patterns had been variables in nutritional and toxicological experiments and not subjects for intrinsic study. It followed that he had had to depend almost entirely upon the contributions of others, and it might be added that, in view of the nature of today's discussion, the choice of material had of necessity been selective.

He was privileged to have had the opportunity of editing since its inception the scientific journal that was now the Anglo-American publication, *Animal Behaviour*, and to have acted during July, 1949, as Joint Secretary of the Round Table Conference on nomenclature in animal behaviour at Cambridge. "Définition de nos termes" was important in any branch of learning, and in the psychological sciences there was ample scope for confusion. Lay usage, or misusage, of words such as "instinct" did not simplify matters, and it was sincerely to be hoped that veterinarians who recorded cases of behavioural interest would do so with due regard to accepted terminology. Dr. W. H. Thorpe had published a report on the Cambridge Conference under the title "The Definition of Some Terms Used in Animal Behaviour Studies" in the March, 1951, issue of the *Bulletin of Animal Behaviour* (No. 9, pp. 34-40), and his remarks to the effect that "In all the discussion on the more elementary types of learning there is apt to be difficulty owing to the fact that the terms which are convenient for the physiologist tend to be misleading or clumsy for the worker dealing with the behaviour of the whole animal" were salutary.

He was indebted to his new co-editor of *Animal Behaviour*, Dr. Larry Weiskrantz of the Department of Psychology, Cambridge, for some extremely helpful comments on the paper as circulated. Dr. Weiskrantz had rightly challenged the use of the adjective "pensive" in describing the appearance of puppies prior to urination midway through the play period. It was easy even for one who was used to checking for such errors to use words in an anthropomorphic sense, and he had fallen into the same trap in writing of the "feelings" of a predator. Dr. Weiskrantz doubted also the assertion of Scott that puppies allowed to run wild until about 12 weeks of age could subsequently be trained, albeit with difficulty, while he disliked the phrase—again attributable to Scott and his colleagues—"biologically maladaptive". This came close to saying that the behaviour was "unbiological", and if in fact it meant the same as "without survival value", then there was little evidence that the behaviour described could not be shown to favour survival of the stronger members of the species. Here again, it was a question of imperfect terminology: what Scott and his colleagues had described were examples of social outcasts in dog communities, and there was little doubt that such animals existed. Those concerned with the breeding and training of dogs were all too familiar with the unfortunate problems. One of the local medical officers of health, Dr. McKellar, who was also a breeder of Boxers, had told him of "ganging-up" problems even among small groups of this breed, and that among them a bitch might be the subject of aggression by males as well as by other females. In the case of Krusinskii's work, he had had to depend upon translations of the original, and could not therefore comment adequately upon the degree of "strength" of the nervous system in his dogs. He should,

however, have written in more detail about the differences between "the classical conditioned reflex of the Pavlov school and trial-and-error learning". As Thorpe (1956) had stated: "The conditioned reflex is, then, best regarded as a minute and artificially isolated piece of the learning process of the animal, and in the isolating procedure it seems that something important is lost. But the conditioned reflex is the basic element of that part of learning which consists in adjusting the innate releasive mechanism to the complexities of the external world . . . we must not expect to find that the simplest learned responses of the whole animal under natural conditions can ever be equated with a simple conditioned reflex and nothing more"; and again, "Another assumption which has proved to be erroneous is that made by many workers . . . that in the classical conditioned reflex the action pattern always remains unchanged irrespective of the unconditioned reflex which serves for reinforcement. It is now recognised that conditioned and unconditioned responses are probably never identical, and that the one is not simply a duplicate of the other. Because Pavlov measured only the salivary secretion and neglected any precise measurement of motor behaviour—in fact, because he did his best to eliminate it altogether—this essential fact was obscured".

A further point suggested by Dr. Weiskrantz related to the case of aberrant behaviour in the young Alsatian described by Joshua, namely, that there must have been something about the garden discriminable to the dog of particular significance, whether or not it was the electric railway.

He wished to record further thanks to Miss Joan Joshua. At the risk of appearing ungallant, he might say that it was some 27 years since he had come to know Miss Joshua. This had been in their pre-student days, and from her he had learned a good deal concerning dogs and their husbandry. Today he found that he could still learn from her, and the inadequacies of his paper would have been considerably greater but for her valuable contribution.

The Opener

Mr. W. B. Singleton, who opened the discussion, said that the author was to be congratulated on the way in which he had reviewed the available literature on the subject of abnormal behaviour of dog and cat. He thought he was right in saying that this was perhaps the first time that this subject had been considered by a totally veterinary audience, and although a vast amount of the paper dealt with the normal, it was quite obviously at this stage very vital that they should have a clear understanding of what was normal and was constituted abnormality. Mr. Worden had dealt with the subject extremely thoroughly, and he was to be congratulated upon his paper.

While he would agree that the study of animal behaviour was of much greater economical importance in species other than the dog and cat, nevertheless a clear understanding of fundamental canine and feline behaviour and the natural response to various conditions and circumstances should be of growing interest to clinicians and others who worked daily with these smaller species. That this study was important was emphasised by the fact that many small-animal practitioners today were becoming generally more successful as clinicians not because they had greater powers of observation but because they endeavoured to confirm diagnosis more and more by laboratory, radiography and other, for want of a better term, "artificial aids". Again, modern therapeutics, with their wide range and application, helped the clinician to obtain better and better results. The danger of those facts was obvious—they might tend to become casual and less and less observant, not of major symptoms, but of the finer points which produced a deviation from normal, either physically or mentally. This was particularly so when they were faced with a psychosomatic case, and let him say quite definitely for the sake of the doubting Thomas's who would probably be present today, that such cases did exist, but careful observation was often required in order to detect them.

The line between normal and abnormal behaviour was not easy to define, and many of the manifestations of what they believed to be abnormal behaviour might be due to environmental factors, particularly if those changed in the early

stages of mental development. Some behaviour which they now considered to be abnormal might be inherent behaviour patterns which were basic for the species or breed.

Mr. Worden's reference to Iljin's observation was interesting, particularly in connection with the point that barking-howling as opposed to barking went hand in hand with domestication. This was certainly in keeping with his own observations of the pack beagle which did not bark and was one of the most difficult dogs to domesticate, whilst the American beagle which had now been domesticated, barked. Continuing the analogy, it probably followed that the more domesticated the dog, the further it went from howling to the yap of pekes, terriers, etc.

While on this subject, it was easy to see, if one accepted Lorenz's view about the domestication of the dog, why a dog now barked; but what was the true purpose of the wolf's howl, and why did some domestic dogs revert to howling at night or when the moon was full or when a particular musical note was struck? Did we humans now consider such behaviour to be abnormal?

Did we tend to blame in-breeding too much as a cause of unfortunate nervous manifestations? He believed that they did—it was not so much a question of in-breeding as lack of culling.

The financial aspect of dog breeding was now very important. It was an industry, and many dog breeders appeared to stand with pad in hand, as a bitch was whelping, furiously assessing their profit as each puppy was born. Of course, if a veterinary surgeon was in attendance he was also standing and probably assessing his profit too! Each pup was given a potential value, and it became an obsession with most breeders to see that every puppy survived to an age when it could be sold at an optimum profit. Even though a puppy was poor physically or had a nervous disposition, too few breeders appeared to realise the harm that such puppies could do to their own breed. Taking a hard and unsentimental view, many of those puppies should be culled, but only the few really genuine breeders today appeared to have the courage to put their practical process into effect; consequently, those indifferent specimens were "protected" until many reached the age of reproduction. It was quite obvious that those individuals, which he suggested were very largely responsible for the abnormal behaviour which they observed in practice, would not survive the natural hazards of a wild state. Consequently, many breeders, from one motive or another, were seriously affecting the basic principle of the survival of the fittest and the balance of nature.

Until breeders took their heads out of the sand and faced the facts, the mental and physical standard of many breeds of dog would continue to decline. Although he sympathised with the practical difficulties involved, he maintained that it was high time that the Kennel Club took active steps to safeguard the mental and physical standards of breeds. To the best of his knowledge, they were not even giving the matter serious thought.

Turning again to the points which the author mentioned in his paper, there was little doubt that in the highly domesticated animal such as the dog the basic instincts of maternal affection and suckling had an influence upon the behaviour of some individuals in later life. Suckling gave a sense of security to both human and canine infants, and the result of this was often apparent in the poodle, for instance, which frequently showed vestigial mouth movements when under stress. Recently he had operated upon a rather temperamental miniature poodle aged 6½ years, which shared a home with its mother. The stress of the operation and the mental effect upon that poodle had caused it to attempt to suckle its dam. This behaviour had gradually disappeared as time passed and recovery from the operation took place. They might consider the behaviour to be abnormal, but was this abnormality when the manifestation was based upon a basic instinct? Mr. Worden's observations upon house training were good and sound. As veterinary surgeons were frequently asked for advice in practice, he felt that the author's observations were well worth repeating. Owners would be well advised to accept the basic pattern as described by the author and use it as a sound way of training their puppies. Certainly the problem of a puppy urinating in the house in one place only and

refusing to pass water anywhere else was a great problem. He supposed that basically it was a marking out of limited territory. However, it was quite a problem in that many of these puppies, even though they were walked for hours and hours in the evening by their owners, would still perform on the doormat, and the only way of breaking this form of conditioned reflex was to take them away for 2 or 3 weeks from the particular houses in which they were living.

Mr. Worden mentioned the work of Scott and her colleagues, who divided the developmental period of puppies into 4 periods: neo-natal, 1-10 days; transition, 10-20 days; 3 weeks to 7 or 10 weeks period of socialisation, followed by the juvenile period. Of these, he felt that the period of socialisation was perhaps the most important. Scott had observed that pups which were not handled during this period were frequently nervous. This was borne out in the human, where it had been shown that babies which were given great affection during early life in the form of cuddling, maternal warmth and comfort were more contented and thrived better than those which did not receive this attention. He had observed that puppies coming from a breeder who devoted time to handling and caressing the pups were much more normal in behaviour and less likely to be nervous than those from a breeder who was too hurried to do other than attend to elementary hygiene and feeding.

Terbergen's studies of territorial behaviour in Eskimo dogs threw interesting light on the savage dog which one met in practice. It seemed that sexual maturity coincided with territory defence; taken a stage towards domestication, it was possible that the abnormally strong drive to defend a home or an owner or even an inanimate object was linked with sexual activity which might be particularly strong in the male—hence the dramatic and often highly successful results achieved by castrating these cases.

As far as early aggressiveness of puppies was concerned, he would not agree with Miss Joshua's view that this should be corrected at an early age. Apart from being a very difficult thing to correct in practice, he felt that the views of Humphrey and Warner that a moderate amount of aggressiveness was desirable were borne out in practice.

He wished to consider briefly what was probably the most common form of abnormality which they met with in practice, namely, nervous anorexia. This was particularly common in the puppy which left its mother and initial environment at an early age, e.g., 6 to 9 weeks. Also, it was more prevalent in the timid puppy. Here two points arose—the advisability of encouraging breeders to handle and play with their pups from an early age and to "bring on" any puppy in a litter which appeared to be of a retiring or nervous disposition, and secondly to encourage individual feeding of pups once they were completely weaned so that the conditioned reflex of communal feeding was broken at an early age—this he believed to be of considerable help in settling a puppy into a new home. Conditioned reflexes and extraneous factors having a direct bearing upon the mental processes of puppies and adults certainly had a very strong influence over a puppy in connection with eating, and might have an effect which transcended the physical need for food. Such cases responded well to tranquillisers in many cases, and sedatives such as phenobarbitone would have the effect of promoting appetite in practically all cases, although the treatment was limited in application because of the undesirable side effects of such drugs.

Nervous anorexia in the adult was more difficult to understand and to treat. Mrs. Brown's dog would not eat unless she powdered her nose at the same time or unless the kitchen door was left open. As a boy he well remembered a neighbour whose dog would not eat unless the wife scrubbed a floor—the house was immaculate. He had often wondered how much the conditioned reflex established so firmly in the dog was established in the other, and what would have happened if the conditioned reflex in the dog had been miraculously broken—would the owner have gone on scrubbing her floor?

This really brought him to the next very important aspect of canine behaviour which he felt warranted discussion today—the influence of a psychiatric owner upon her dog. Unfortunately, he was not a student of human psychology, and

he did not pretend to understand the inter-relation and interaction which occurred under certain circumstances between dog and owner. Nevertheless, it was obvious when one considered a few bare points that a deep and far-reaching association could develop given the necessary circumstances. First of all, there was the shifting of maternal affection of a puppy from its own mother to the two-legged one which could produce a strong connection from both points of view. In the "wolf type" dog there was in addition the inherent instinct to accept a "pack leader" which normally took the form of the human owner—thus a strong bond was created between the two. On the other side, female owners under certain circumstances responded readily to the "maternal" demands made upon them by the lonely, helpless puppy. What many people would call an unnatural association developed, the bonds of which were extremely strong. This was particularly likely to occur in the female owner who was childless, lonely, and who lacked masculine affection, and particularly if such a person lived a relatively empty life.

Thus a completely unbalanced association developed, the strength of which should never be underrated by the small animal practitioner, who should always try to deal with these circumstances with tact and understanding—not always easy in the hectic harassing days which modern life demanded of them. However, it was not difficult to appreciate that the interaction between dog and owner under these circumstances occurred readily, and nervous tension on the part of the owner was transmitted to the dog and was manifest in several ways as, for instance, nervous anorexia, abnormal timidity, and psychosomatic manifestation, such as scratching and anal gland irritation unrelated to direct physical causes. Again, such strong relationships produced mutual possessiveness which made the dog highly nervous, snappy and difficult for a stranger to handle unless removed from the presence of the owner, when they invariably became submissive and docile.

He had purposely kept his remarks general because to quote individual examples of abnormal behaviour was probably fairly easy to do, but tended to become boring after a while.

The General Discussion

Dr. F. R. Bell (R.V.C., London): "I should like to take this opportunity to congratulate Mr. Worden on reviewing this difficult subject so admirably, and Mr. Singleton for correlating the paper with the types of abnormal behaviour that are met with in veterinary practice today. There is one aspect of this problem to which the essayist has not made reference, namely, the inter-relationship between the endocrine system and the central nervous system. An example of this inter-relationship is the effect of the gonads on affective behaviour patterns. If an adult male dog is castrated then he ceases to cock his leg to urinate and reverts to the female or juvenile pattern of squatting and of expelling the urine from the bladder in a single large volume. This type of behaviour reaction can be reconverted once again to the usual male act by the injection of androgen. This example illustrates the very close interdependence of the various systems that have to do with overt behaviour. It is quite possible that this effect of hormones impinges on the central nervous system at various levels but it is likely that the hypothalamus may be the co-ordinating link."

Mr. N. Comben (Southall): "Much that has been said in this discussion about dogs applies equally well to cats. In particular, I refer to the different behaviour of many pet animals in the environment of their own homes to that exhibited in the confinement of unfamiliar kennels and catteries. We have all had experience of the animal which, though docile and easily managed at home, becomes ferociously aggressive on confinement in a cage. Such an animal frequently suffers complete nervous anorexia at the same time.

"These changes may be more marked with some breeds of cats—in particular the Siamese—than others. At a recent meeting of the Metropolitan Division of the B.S.A.V.A., where the peculiarities of this breed were discussed, I suggested that it is simply because of the more pronounced

lowering of general condition and weakened resistance to infection which followed such changes, that the Siamese becomes particularly susceptible to certain virus infections in catteries. I do not believe that this breed is any more susceptible in the first place to these infections than other types of cats in their normal home environment—however bad patients they may be when they are ill.

"Such changes in the behaviour of pet animals can affect—and often make more difficult—our work with them, and any study of this nature must always be related to its application in general practice."

The Reply

Mr. A. N. Worden, in reply to the discussion, thanked Mr. Singleton for his opening remarks and for his affirmation that psychosomatic cases existed. With regard to Mr. Singleton's statements about difficulties of domestication with the beagle and the difference between British and American beagles, he thought it was most important to realise that these periods of development were of vital importance and that in fact at different stages dogs of inherently different trainability in different ways might be picked out. On the question of why domestic dogs howled, he thought that clearly some situation acted as a releaser for that type of howling, which was, of course, an innate mechanism. As for the miniature poodle which at the age of 6½ years endeavoured to suckle its mother under the stimulus of some reinforcement, he imagined that this was not so much a case of abnormal behaviour as an instance of normal behaviour being called into play by circumstances, but probably for 6½ years prior there had been an abnormal relationship in terms of pack between the two dogs. He imagined that these releaser situations must be responsible for behaviour of that type, but also for the forms of aberration which sometimes concerned things like sheep worrying and the killing of other animals, and so forth.

Various speakers, including Mr. Singleton, had referred to the desirability of a moderate degree of aggressiveness or forward behaviour in pups. He thought that most workers would agree with the majority view this afternoon that some degree of aggressiveness, provided it was not too great, was highly desirable for future training, and Lorenz believed the same to be true of cats as of dogs. Miss Joshua had pointed out that there was a time at which the pup should be taken in hand, and he reinforced the remarks made in his paper that one of the best forms of chastising a dog of any kind was not to hit or strike or kick it in a way that it did not really understand, but to shake it by the scruff of the neck.

Mr. Singleton had mentioned nervous anorexia and the difficulty of explaining its occurrence and the even greater difficulty of treating it in an adult as opposed to a puppy. He thought that possibly the main factor in these cases must be some breaking of the normal behaviour patterns. Usually these cases tended to occur either under the stimulus of kennelisation or some change, perhaps unnoticed to the owner, or routine within the home. Mr. Singleton had referred to woman/dog relationships, and these had been discussed further by Miss Freak. Obviously one was here dealing with a combination of possibly two sets of unsatisfied drives, and the combination could be deadly.

Dr. Bell had referred to the alteration of the pattern of the normal urination of the male dog under castration and its restoration with replacement therapy, and Dr. Cotchin had referred not only to the production of a feminine type of urination posture, but also to his own experience of a dog which he walked home which urinated 40 times and once failed to lift its leg. Possibly it got tired the fortieth time! But he thought that this had been almost certainly related to the confinement to other quarters, and he only wished that in the dog all effects could be explained away in relation to castration and to male hormone as, apparently, those of the urination pattern. As indicated in the paper, the effects of castration upon male dogs were extremely variable and in many cases unpredictable.

Mr. Bishop had referred to kennelisation and to the various effects of transferring dogs to such surroundings and confining them there. Of course, such dogs were often placed

in a very much more restricted territory than that to which they had been accustomed, and they were, of course, also faced with new surroundings and new reactions from them. Mere size of cage or other area in which an animal was confined had an effect on urination. They had found experimentally with dogs put into small cages that they refused to regard such a cage as territory but sat there and held their urine.

With regard to the male that appeared to become impotent or disinterested in the female after a long confinement to kennels, he thought that there was an analogy here with human experience. Despite examples to the contrary, there were reports of soldiers and other service men who had been kept entirely in male surroundings for some months or years during the war and who had in fact become impotent or completely abnormal in their relation to the opposite sex. He felt that one should perhaps think of kennels as one thought of armies or prisons in any comparative study.

Mr. Bishop's remarks reinforced the view that most dogs, at any rate, were expected to mate in highly abnormal circumstances without any prior attempt to provide conditions of build-up or of understanding the situation, and it was quite remarkable to him that there were not more failings in such matings than actually occurred.

Miss Freak had referred to the risks of carrying out a caesarean section in bitches of the hunt kennel type and the subsequent failure in such cases for normal maternal behaviour to be expressed. Miss Brancker had suggested that perhaps those circumstances which led a caesarean operation to be necessary were responsible also for the lack of maternal drive. That view might certainly be correct. He thought it was probably much more dangerous to interrupt the drive in a less socialised animal than in the case of an animal which had a more stereotyped behaviour, and he would have predicted that an animal in hunt kennel conditions would be less able to withstand interference than the animal used to family life.

Miss Freak had referred to the quasi-male behaviour of the female during urination and occasional attempts to cock a leg, and so forth. He thought that this had probably been seen under various conditions. He had seen it in cir-

cumstances where he thought it was purely an imitation of the male dog.

Miss Brancker had referred to one male whose highly active sex drive had been expressed only in respect of good-looking bitches. He could only say that one must avoid comparative interpretations!

He was sorry that he had used Miss Joshua's communications almost verbatim, but in fact he was very grateful to her for the very great help she had given him; it had enabled him to provide a paper of some sort of practical as well as theoretical interest. He agreed with Miss Joshua in her emphasis that the basis of training should be natural behaviour patterns—and not only for house training. Almost all successful forms of training for a dog as a working dog really depended upon the same principles—or should do so.

Mr. Comben and Dr. Scott had referred to the cat, and Dr. Scott had remarked that the cat was even more human-dependent than the dog. He would agree with that, but he would say that possibly the nature of the relationship between cat and human being might differ somewhat in that the cat behaved less like a pack animal than the dog. When cats were run together in groups their form of behaviour was somewhat different from that of dogs run together in groups, and he suggested that the same sort of factors operated in cat-human relationships.

He did not think that anybody had made a precise study of the development of the kitten, or at any rate had not pinned it down so precisely as Scott and his co-workers had done for puppies, and it might be that the periods were slightly different in the kitten and the puppy.

Mr. Comben had referred to upsets of animals in quarantine and in altered surroundings and to the fact that in some cases visits by the owner put the animal off food to which it had become accustomed. Again, one was tempted to use comparative explanations. This behaviour tied very closely with the behaviour of a child in hospital or a child sent to a boarding school for the first time. However, he did not wish to introduce a comparative element into the paper, since comparisons were dangerous, and obviously there was a great deal more work to be done on all aspects of this subject.

APPENDIX III

Some Aspects of the Nutrition of the Dog and Cat*

BY

A. N. Worden : Patricia P. Scott : R. G. Booth

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I.—The Dog

BY

A. N. WORDEN

Nutritional Research Unit, Huntingdon

IT is now over 15 years since the valuable review of the nutritional requirements of dogs by Michaud & Elvehjem (1944) was published, 10 years since the appearance of the second edition of McCay's (1949) text-book, and 5 years since the National Research Council's (1954) report on the nutrient requirements for this species was produced. A great deal has happened even since the last of these, and it would be impracticable in the time available to try to bring relevant knowledge up to date. Partial coverage of the field has been made by Bourne (1956) and by Schwick (1959), and it is proposed that this evening a few selected topics only should be dealt with.

Of general interest has been a study of drying conditions on the nutritive value of a processed stock diet designed primarily for dogs (Harms & Scott, 1956). A diet containing fish, whalemeat, meat-and-liver meal and biscuit waste, with propyl gallate as antioxidant, supported good growth in weanling rats after it had been dried in air temperatures below 140°C. The same diet, after drying at 140° or 150°C., produced fluctuating gains, and after drying at 160°C. led to anorexia, stunted growth, poor coats and reproductive failure. These symptoms were thought to resemble those shown by rats fed diets deficient in protein or in amino-acids such as tryptophan. There was some reduction in thiamine and vitamin A in the diet dried at 160°, as judged by chemical analyses, and possibly of other vitamins also, although the symptoms did not resemble those of any specific vitamin deficiency. The authors believed that the poor results, at least in the diet dried at 140°, were due principally to failure to digest the protein that had become "damaged" at this relatively low temperature by the formation of *N*-glycosides in the presence of reducing sugars.

Damage to, or inactivation of, amino-acids through processing is, of course, a widely recognised phenomenon, and in the case of many proteins it is the available lysine that becomes reduced through coupling of the ϵNH_2 group to form an amino-sugar. Special methods are now available for the chemical determination of available as opposed to total lysine. The last figure may be misleading and give a faulty index of the nutritional value of the protein. Even microbiological assays, with their attendant extraction procedures, give total as opposed to available lysine. For proteins intended for feeding to growing animals, including puppies, a satisfactory supply of available lysine is essential.

The beginning of the present decade saw what

would appear to have been final clarification of the canine hysteria problem as related to the use of biscuits, bread or other farinaceous materials prepared from agerised flour. In experiments reported subsequently to those referred to at the forum meeting of this Society in 1950, methionine sulphoxamine was found to provoke typical symptoms in puppies. The prior administration of methionine itself was found to be protective, but once the signs were established methionine was without effect (Gershoff & Elvehjem, 1951).

The Feeding of Puppies

Puppies that have been used to feeding together will take more food in the presence of their fellows than when fed separately. Puppies that have been fed separately, even though reared together otherwise, do not for some time consume more food when fed together. In the studies reported by James & Gilbert (1955), the effect did not become significant until after some 14 days of alternate separate and group feeding.

In Swedish experiments on the artificial rearing of puppies it was found that excellent results were obtained with a mixture comprising: whole milk, 800 ml.; cream (12 per cent. fat), 200 ml.; one egg yolk; bonemeal, 6 g.; and citric acid, 4 g.; together with 2,000 I.U. vitamin A and 500 I.U. vitamin D. The puppies were caged individually, kept warm with lamps, and fed 6 times daily. The daily allowances of the mixture, as percentages of bodyweight, were 15 to 20 at 3 days of age; 22 to 25 at 7 days; 30 to 32 at 14 days of age; and 35 to 40 at 21 days (Björck, Olsson & Dyrendahl, 1957).

Puppies fed casein as the sole dietary protein have been found to retain somewhat less nitrogen than those fed whole egg protein, but more than twice as much as those fed wheat gluten. Nevertheless, animals on all 3 sources of protein gained in bodyweight at about the same rate, the increase per g. nitrogen intake being 9.0 g. for whole egg, 9.8 g. for casein and 8.8 g. for wheat gluten. There were, however, notable differences in behaviour, appearance and body composition. The animals fed wheat gluten were low in body protein but high in fat stores compared with those fed on egg or casein. Puppies fed egg protein were lean and active while those fed wheat gluten were obese and more lethargic. As Allison & Wannemacher (1957), who deal also with the repletion of depleted protein reserves in adult dogs, state: "these and other observations on growth or repletion of tissues emphasise the importance of establishing an optimal relationship between caloric and protein intake to keep a proper balance between fat stores and lean body mass."

Total Food Requirement

Recent studies on the total food requirement of dogs serve to confirm earlier findings and to indicate also the wide individual variations in voluntary con-

sumption. In a series of observations involving Alsations, Salukis and Basset hounds, it was found that voluntary food intake ranged from 1 lb. per 20 lb. to 1 lb. per 50 lb. bodyweight daily, and was independent of breed. After 20 weeks on a diet supplying 1 lb. per 36 lb. bodyweight daily, all animals were in good condition and had received adequate food for maintenance purposes. The digestive efficiency at this level was about 80 per cent. Dogs having the largest appetites were the most active, but age and activity appeared to have little correlation as did weight and food intake within the range observed (James & McCay, 1950).

The effect of age was further studied in Beagle bitches ranging from 3 months to 12 years old. It again appeared that older animals could digest the dry matter of their ration as efficiently as younger ones. Young puppies, however, digested the protein of a low-protein ration, and the fat of a high-fat ration, less efficiently than adults (Lloyd & McCay, 1955).

Calorie : Protein Ratio

There seems little doubt, from the studies just referred to and from many other findings, that a correct calorie : protein ratio is of importance to adult dogs as well as to puppies. Nitrogen is wasted, as in other species, through restriction of caloric intake or through relative excess of dietary protein. Rosenthal & Allison (1951) studied the effect of restricted calories in experiments with adult Fox Terriers receiving a constant casein nitrogen intake of 3.82 g. per day per square metre of calculated body surface. Calories were varied by altering the intake of carbohydrate. In confirmation of earlier studies, 2 responses were recognised and, to quote from their summary: "the first response, obtained with even a slight reduction in dietary calories, was an increase in the excretion of urinary nitrogen without any change in the nitrogen balance index of the dietary protein, a response which reflected an increase in the catabolism of proteins. Continued feeding of a markedly restricted intake depleted the protein stores of the body. The second response followed the first in the presence of a severe caloric restriction and was dangerous to the animal. Some animals resisted the second response much more than others, a resistance which was correlated, at least in part, with the magnitude of the protein stores of the body. It is emphasised that responses to a restriction in calories vary with the physiological state of the animal and with the composition of the diet fed."

In our own studies on diets for sledge dogs, preparatory to the Trans-Antarctic Expedition of 1957-8 (Taylor, Worden & Waterhouse, 1959), we found evidence of faulty utilisation of beef pemmican, a dried and concentrated food made mainly from beef and consisting of about two-thirds protein and one-third fat. The analysis of the faeces from Huskies in the Antarctic indicated that about one-third of the protein and about 6 per cent. of the fat were excreted in the faeces. Studies in metabolism cages showed that during pemmican feeding there were abnormal urinary changes, retention of water, indicanuria, and excretion of fluorescent substances. There was reduction also in the output of thiamine and of riboflavin.

The animals themselves sometimes lost weight and tended to develop loose stools. Substantial improvements, in the laboratory and in the field, were obtained when the calorie : protein ratio was adjusted.

Lipids

Fat is usually present in formulated dog foods. Typical formulations conforming to the standard for canned dog foods, agreed some years ago by the Institute of American Meat Packers, would contain about 8 per cent. fat on an original basis and have a calorific value of between 600 and 700 calories per lb. By contrast, comparable cat foods would have a fat percentage of only about 4, and a calorific value of between 500 and 600 calories per lb.

The utilisation of the calories of fat was found to be quite satisfactory in experiments with growing Cocker Spaniel puppies: there appeared to be equal utilisation from 4, 6 or 8 per cent. added stabilised fat and from isocaloric additions of sucrose to a basal ration comprising flaked maize, flaked wheat, soya bean, meat-and-bone scrap, fish meal, wheat germ, skim milk and vitamin supplements. The addition of 6 per cent. fat to a commercial dry dog meal was similarly beneficial (Seidler & Schweigert, 1952). In further experiments (Seidler & Schweigert, 1954), the addition of 4 or 8 per cent. fat, or of 18 per cent. sucrose, was found to increase the efficiency of a basal ration for maintenance of females prior to breeding, and at the 4 per cent. level there was some improvement in reproductive performance. The addition of 8 per cent. fat to the basal ration, however, appeared to lower the reproductive capacity of puppies in that, although more were born, the birth weight and the viability at 24 hours were lower.

Much higher levels of fat have been used for growth purposes. As would be predictable from earlier fundamental studies on the dog, and from the use of high-energy rations for broilers and other productive animals, at a dietary fat content as high as 30 per cent., the growth of weanling puppies has been found to improve with dietary protein level from 20 up to 29 per cent., although at 33.3 per cent. protein efficiency is no better (Ontko, Wuthier & Phillips, 1957). In these experiments larger puppies were more sensitive to faulty protein : calorie relationships than smaller ones. This again would have been predictable on general grounds. An extremely high level of fat, *i.e.* 40 per cent. of the diet, has been reported to cause a greater loss of calcium in adult dogs than a level of 5 per cent. (Liu & McCay, 1953).

A possible harmful effect of a high fat intake in very young puppies has been reported by McIntock & Wilkinson (1954). Severe ataxia, nodding movements of the head, muscular tremors, diminished pedal reflexes, bloated abdomen and breath smelling of acetone was found in all 6 members of a litter of 4-week-old Samoyeds.

Work during the past 2 decades on fat as a dietary essential for the dog was discussed at the symposium on non-parasitic skin diseases in dogs and cats held by this Society in 1957 (Worden, 1958), and some aspects on a previous occasion, the "forum" relating to the nutrition of the dog held in 1950 (*Vet. Rec.*, 1952, **64**, 232). Reference was made at the symposium

to the extensive studies on experimental fat deficiency in the dog by Hansen and his colleagues (Hansen & Wiese, 1943, 1951; Wiese & Hansen, 1951 a, b; Hansen, Holmes & Wiese, 1951) and to the circumstantial evidence that fat-deficiency occurred in dogs during the second world war (Joshua, Iszard & Worden, 1945).

Parenteral feeding studies have indicated that dogs remained healthy and without loss of bodyweight if a significant proportion—in this instance 34 per cent.—of the total calorific intake (of 80 calories per kg. bodyweight daily) was derived from fat, but not when this fat was replaced isocalorically by glucose. Isocaloric replacement with glucose together with the methyl esters of linoleic, linolenic and arachidonic acids was, however, reported as satisfactory (Meng, Youmans, Cain & Grimes, 1953).

There has not, however, been general agreement on the role of these so-called essential fatty acids (E.F.A.) in species other than the rat, and in man it remains a vexed question whether these E.F.A. are responsible for the effects on blood cholesterol levels of certain unsaturated fats, or whether the activity is due to some other properties of the unsaturated fraction. Current evidence favours the latter, but the dog must be studied in its own right.

The effect of diet upon blood cholesterol and upon atherosclerosis has been studied by a number of workers (including Bevans, Davidson & Abell, 1951; Shull, Mann, Andrus & Stare, 1954; Barr, Russ, Eder, Kendall & Abell, 1955; and Marmorston, Rosenfeld & Mehl, 1959). Shull *et al.* found a very varying blood cholesterol response to a high dietary cholesterol, but that even with prolonged elevation of blood cholesterol and lipoproteins there was no arteriosclerosis. The other workers have fairly consistently reproduced atherosclerosis from a combination of cholesterol and thiouracil, or by total thyroidectomy followed by a high cholesterol diet. Marmorston *et al.* have described a marked degree of involvement not only of the coronary vessels, but also of the cerebral arteries and of most of the smaller vessels of the body.

Calcium

The recent important studies by Scott and her colleagues on cats will presumably be discussed later. An osteoporosis, associated with flesh-eating habits, occurs in other carnivora and Scott (1959) has expressed considerable doubt on the contention that *osteogenesis imperfecta*, of the kind described in a 10-week-old Collie bitch by Holmes & Price (1957), is of genetic origin. It would seem much more likely that nutritional factors, of the kind operative in cats, are responsible.

There is certainly need to check the calcium (Ca) intake, and the calcium:phosphorus (Ca:P) ratio in dog diets. This was made clear in the studies of Morgan and her colleagues over 25 years ago (Morgan & Garrison, 1933; Morgan, Garrison & Hills, 1933; Morgan, 1933). These workers showed that high calcium (230 to 740 mg. per kg. bodyweight daily) combined with low phosphorus (90 to 140 mg. per kg. daily) diets, in which the Ca:P ratio was from 1.6 up to 8.2:1, were rachitogenic in the absence of vitamin D, whereas a low calcium and high phos-

phorus ration, gave rise to a characteristic osteoporosis. In one experiment this condition, described as *osteitis fibrosa*, arose from the feeding of a diet supplying only 100 mg. Ca per kg. but 1.02 g. P per kg. bodyweight daily, a Ca:P ratio of 0.1:1. The young dogs recovered when given adequate calcium lactate. In their experiments in which the Ca:P ratio was nearer to unity (over Ca intakes of 360 to 800 mg. Ca and 420 to 570 mg. P per kg. bodyweight daily, giving Ca:P ratios of from 0.7 to 1.4:1), calcium and phosphorus retention was nearly the same whether or not vitamin D was added.

Later studies, in so far as they have taken these basal findings into account in the experimental design, are supportive. On low Ca intakes (4 to 8 mg. per kg. bodyweight daily) dogs of from 6 to 25 weeks of age all show negative Ca balances, there being no age at which losses are at a minimum. In older animals, of from 6 to 15 years, there is difficulty in maintaining Ca equilibrium unless at least 86 mg. Ca per kg. is supplied daily (Liu & McCay, 1953). In studies on fresh bone it was shown that, on the same basal diet, Ca retention in growing dogs fell from 56 per cent. at 80 days of age to 26 per cent. at 200 days and to 20 per cent. at 330 days. In the same animals retention of P was similarly 56 per cent. at 80 days, fell to 30 per cent. at 120 days, but had risen to 45 per cent. at 330 days (Udall & McCay, 1953).

Recently Gershoff, Legg & Hegsted (1958) have studied the adaptation to different calcium intake in dogs. During the period of rapid growth, 90 per cent. of Ca in a diet containing 0.114 per cent. Ca, 46 per cent. of that in a diet containing 0.634 per cent. Ca, and 27 per cent. of that in a diet containing 1.234 per cent. Ca, were retained. As the dogs grew older, those accustomed to a low Ca intake appeared to remain in positive balance even on a low Ca intake. Andreasi (1955), in his studies on the feeding of a commercial dog meal to Beagles, found better retention of Ca, P and nitrogen at 12 weeks of age than in adults.

The view has been expressed that urolithiasis in dogs is a disorder of Ca and P metabolism. An analysis of the records of urinary calculi, conducted at the Royal Veterinary College, Stockholm, indicated a much higher incidence in chondrodystrophic breeds, such as Dachshunds and Pekingese, than in Airedales, Alsations or Boxers (Krook & Arwedsson, 1956). It must, however, be emphasised that cystine calculi are not uncommon in some of the chondrodystrophic breeds, as well as in Dalmatians (see Bloom, 1954), and that the evaluation of any single factor must be regarded against the whole background.

Water

Over 30 years ago it was reported that there was great variation in the quantity of water consumed by individual laboratory dogs from day to day. The animals in question were not normally fed on Sundays, and on these days their water consumption was small. When the dogs were deprived of food for a number of days in succession, they drank more water daily than on foodless Sundays, but appreciably less than during days of normal feeding (Kleitman, 1927).

From the data in Kleitman's paper it may be calculated that, for dogs weighing 8 to 14 kg. (17½ to 30 lb.)—within which range consumption did not appear to be related to bodyweight—the mean consumption on weekdays averaged 352 ml., that on Sundays only 64 ml. and that during periods of fast 148 ml. The total water intake by the body was of course even lower during fasting, owing to lack of water obtained from the food itself, and Kleitman calculated it as from one-fifth to one-third of the normal. He believed that the marked decrease in the water intake in starvation was probably one of the causes of the gradual deterioration of the salivary conditioned reflex observed in that condition.

Despite this lowered intake in starvation, limited studies have indicated a marked increase in the antipyrine space of emaciated compared with normal dogs (Herrold & Sapirstein, 1952).

In very hot weather, especially out of the shade, water consumption may be doubled (Whitney, 1949, p. 81). According to Tarbin (1955) dogs consume larger volumes of water daily at higher environmental temperatures by taking more frequent drinks rather than by any change in the amount of water taken at each drink. A given dog appears to favour a characteristic size of drink that is not markedly affected by total food or water intake. As mentioned at Congress (Worden, 1959) adult dogs with kidney damage, presumably of the chronic interstitial type, will consume very long single drinks.

Vitamins

Although we have for some years been studying the utilisation and excretion of various vitamins by the dog, this is too lengthy a topic for consideration here. We have confirmed that small quantities of vitamin A are present in the urine of normal dogs (Worden, Bunyan, Davies & Waterhouse, 1955), although the levels (from about 2.5 to 50 I.U. per 100 ml.) are of a lower order than previously reported and we would regard the excretion of large amounts as indicative of a pathological condition.

In our studies on the B group (Worden, Waterhouse & Partington, 1952, 1954, 1955; Worden & Waterhouse, 1955; Worden, Waterhouse & Pulman, 1956) we have evidence of variation of utilisation with environmental temperature and, in the case of thiamine, a marked increase during pregnancy. The lability of thiamine, and its almost complete absence from the beef pemmican diet as used, led to deficiency in the field (see Taylor *et al.*, 1959). Greig (1953) has referred to deficiency of nicotinic acid, as well as of calcium and, possibly, riboflavin and vitamin D, in the regular diets of sheep dogs in Great Britain.

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APPENDIX IV

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FEEDING OF LABORATORY DOGS AND CATS

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DOGS

THE writer has not had the privilege of prior consultation with the other speakers, but it is clear that the papers up to this point have covered or referred to virtually all the theoretical and experimental background to the subject, and that it is necessary to concentrate upon the practical feeding of laboratory animals used for other than nutritional investigations. I am glad that Mr. Walker and Mr. Hodgman have both been so honest in emphasizing the accidents and other unwanted problems that may arise when dogs are employed experimentally.

At Huntingdon we have substantial numbers of experimental dogs to maintain, and shall shortly have over 800 Beagles, Corgis and Cocker Spaniels on chronic toxicity studies alone. The Beagle is the usual breed of choice for this work, and a paper has already been presented by Walker (1964) on the rearing of it on controlled diets. When we first began studies of this type, we were unable to secure adequate numbers of Beagles in this country to make up the uniform groups required, and chose the Pembroke-shire Corgi, which as we have described elsewhere (Worden, Noel and Jolly, 1963) has many advantages, including its relatively uniform rate of growth to an adult weight of approximately 10 kg. It is, however, a solitary animal, and suitable for group maintenance only when adapted to it at an early age, and then for relatively limited periods. The Beagle, however, usually thrives in groups as might be anticipated from its working use. Cocker Spaniels, as my colleague Donald Jolly has found hitherto, will also mix well with its fellows. Smaller numbers of dogs of these same breeds are kept for so-called acute or subacute tests or for metabolic studies. We do not employ mongrels or other animals of uncertain origin even for these, our sole sources of supply being established pedigree breeders.

Chronic toxicity studies tend, at the present time, to conform to a somewhat stereotyped pattern, developed mainly in helping to fulfil the "non-rodent species" requirements of the United States Food and Drug Administration (the FDA) for preclinical testing of drugs and for pre-human exposure of food additives, pesticides and other substances. Many of our studies are to

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meet FDA requirements, while those designed to comply with the regulations or recommendations of this and other countries are similar in scope.

The test materials include not only a wide variety of human drugs, but also food additives, food packaging materials, insecticides, herbicides, cosmetics and toiletry preparations, household and factory chemicals that may conceivably present a hazard to human health, and veterinary or animal husbandry products that can enter into the flesh or milk, or otherwise be consumed or come into contact with man. The dog (or other non-rodent) studies are paralleled by studies on rats or mice, and their duration ranges usually from 3 to 24 months, the majority lasting for 6 months. There are usually 3 test groups and a single negative control group, all 4 groups comprising a minimum of 6 animals, 3 males and 3 females.

The 3 test groups have a range of dosage levels, the highest of which is intended to be an "effect level", i.e. a deliberate attempt to produce a definite symptomatology or pathology and to select which are the "target organs" likely to be effected. In those instances in which profound effects are induced, humanitarian considerations and the provision of the Cruelty to Animals Act of 1912—as reinforced by routine visits from the Home Office Inspectorate—alike dictate that this particular part of the experiment be terminated forthwith although the remainder will continue to the end of the prescribed period. In many instances, however, even massive doses have slight or no effect, or may safely be continued. Indeed, we are often faced with the problem of increasing the highest dosage level several times throughout the experiment in the hope of producing some definite effect.

Prior to administration of the test material, at intervals throughout the experiment, and again at termination, each dog is subjected to a battery of haematological and biochemical determinations. The range varies somewhat but usually includes red cell count, haemoglobin, MCHC, PCV, reticulocyte count, platelet assessment, clotting time, prothrombin time, clot reaction time, total protein, electrophoretic pattern, urea, glucose (after fasting overnight), alkaline phosphatase, serum glutamic pyruvate transaminase, isocitric dehydrogenase (or other enzymes), bilirubin and the usual range of urological procedures.

At the end of the study, each dog is sacrificed and subjected to a detailed *post-mortem* examination, including organ weight analysis, with subsequent histological study of from 20 to 40 or more tissues. It should therefore be feasible to pick up evidence of some forms of gross nutritional defect. Moreover, the dogs are weighed weekly, examined by a skilled technician daily and by a veterinarian at intervals for ophthalmic or other changes, and where the class of compound so indicates are subjected to routine electrocardiographic, electroencephalographic or other procedures.

This comprehensive preamble is necessary to emphasize that at Huntingdon practical feeding problems would not end with the provision of a palatable

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diet that contains the known nutritional requirements of the dog in their correct proportions. First there is the mechanical problem of administering the test substance. With most drugs, and with some other classes of compound, we nowadays usually administer the daily dose by capsule or by stomach tube, or sometimes in a discrete portion of palatable food. In the case of food additives, however, or of food packaging materials or pesticides that would be expected to enter man through contamination of his diet, it is customary to incorporate these in the food at defined concentrations. This may involve considerable pharmaceutical skill, since the physical form may not lead to satisfactory distribution despite the careful use of premixes. For an attempted effect level, or one based upon 100 times or more the maximum likely human dose, quite considerable quantities are involved. We were involved some years ago in studies on an organophosphorus plasticizer which at the high level formed 0.5 per cent of the total diet. As we shall shortly be reporting to the European Society for the Study of Drug Toxicity (Harper and Worden, 1964), admixture with the diet may sometimes greatly lessen or—through the formation of more toxic derivatives—greatly enhance the toxicity of a drug. There is also the possibility that large amounts of extraneous substances may interfere with the absorption or utilization of dietary essentials, and we had some evidence, for example, that the plasticizer in very high doses was lowering vitamin A absorption.

Long-term feeding studies of this type pose quite different problems from those of feeding household or even working dogs, with palatability a critical factor. We are loath to make even a slight change in dietary régime once an experiment has started, and therefore endeavour to choose one that will serve for the whole period. The animals do not as a rule come into the experimental quarters until over 4 months and sometimes—particularly for certain American-sponsored studies—not until they are 6 to 7 months of age, and may not take kindly to radical changes. Adaptation to a so-called complete dry diet is, for example, much easier when it is introduced at or soon after weaning. Although our animal technicians are under skilled supervision and devoted animal attendants, they just do not have unlimited time to spend coaxing and training them to take diets not readily acceptable.

Another complication is the group factor. In many of our experiments the dogs are maintained singly in runs, and although sometimes exercised in groups, this is not at feeding times. To quote from our earlier contribution (Worden, Noel and Jolly, 1963): “. . . puppies that have been used to feeding together will take more food in the presence of their fellows than when fed separately. On the other hand those that have been fed separately, even though reared together otherwise, do not for some time consume more food when fed together.” Exercise is of significance also, even though, to repeat from Abrams' (1962) information that has probably already been given at this Symposium, something like 1 hour's hard exercise daily is needed to

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dissipate the energy furnished by a dietary surplus of 15 to 20 per cent above maintenance provision. As we have observed previously, the additional caloric utilization of running or walking outside the confines of a cage is probably a good deal lower than might be supposed from superficial observation. Moreover, even dogs that are quite closely confined will take a great deal of exercise, especially if there are many of their fellows in the same room. We do not maintain dogs in cages on the system adopted in so many American laboratories with, it would seem, considerable success. The writer has visited such laboratories and observed the dogs over varying periods and am quite satisfied that such animals can, and often do, remain healthy and suitable experimental subjects for periods of up to 3 years or more. Moreover, they appear to be happy and are not overweight and often in surprisingly fit-looking condition. It is claimed that the uniformity among them, in terms of, for example, haematological values, is greater than in animals with indoor-outdoor runs or other more extensive quarters.

Perhaps more important than additional exercise—as such—is satisfaction of inborn or early acquired behavioural feeding patterns which has been reviewed earlier (Worden, 1959, 1960) and to which we shall return in the case of the cat, and which will doubtless be enlarged upon in the final paper by Wackernagel (1965). Possibly the daily walks so much enjoyed by the domestic dog have a similar important role quite apart from exercise as such.

When we discussed practical feeding in the 1963 chapter we were not then in possession of some later information on the use of so-called “dry diets”, for the only ones we had by then attempted to feed for any length of time had failed on the grounds of early adaptation or long-term palatability. We have now, however, experience of others, including 2 that are giving us good results to date. One of these is the Ralston Purina Dog Chow, imported from the United States, and the other the P62 diet made by Spillers in this country, which is the graded larger diet referred to by Walker. Details of the P62 diet, are given in Table 1 and both clearly have adequate palatability and nutritive value to sustain dogs for several months at least as the main diet. The performance of groups of control dogs, both male and female, on these 2 diets shows that the rate of growth, for animals over approximately the same age range, occurs quite well despite the breed difference. There may well be other equally suitable diets, and we shall in fact shortly be trying some of these out. The 2 mentioned are fed on the self-service system from stainless steel hoppers. The routine allowance is 400 g/day/dog up to 6 kg body weight, and this is increased to 600 g beyond that weight. There is, however, individual food recording (or in the case of small groups, group food recording), supplemented by careful observation of the animal, its excreta and its weight curve, and the exact daily allowance is adjusted accordingly. All the dogs so fed receive, in addition, one-third of a pint, or approximately 190 ml, of cow's milk and one Bob Martin's

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TABLE 1. SPILLERS DOG DIET (P62)
TYPICAL NUTRIENT ANALYSIS

<i>Calorific value</i>		1400 cal/lb
<i>Protein</i>	%	
Meat	5.6	
Soya	11.3	
Yeast	0.8	
Cereal	6.6	24.3%
<i>Fat</i>		
Animal tallow	6.2	
Vegetable oil	2.1	8.3%
<i>Minerals</i>		
Calcium		1.3%
Phosphorus		0.9%
Sodium chloride		1.0%
Potassium		0.7%
Magnesium		0.2%
Iron		0.1%
Manganese		30 ppm
Zinc		15 ppm
Copper		10 pp.m
Iodine		2 ppm
<i>Vitamins</i>		
A		4.2 i.u./g
D ₃		2.4 i.u./g
E (as α -tocopherol)		10.1 mcg/g
B ₁ (thiamin)		5.1 mcg/g
B ₂ (riboflavin)		2.7 mcg/g
Nicotinic acid		51 mcg/g
<i>Lysine</i>		
Total		1.9%
Available		1.3%

"Nutricon" vitamin tablet (the composition of which is also given in Table 2) daily. As we have previously stated, a routine vitamin supplement, with previous dietary régimes anyway, has been associated with good coat lustre. We have an open mind upon the need for such supplements with present-day diets but prefer to retain what we regard as a wise safeguard. Reference should be made to the contributions presented by Tagwerker (1965) and by Scott (1965).

Some of our long-term experiments were started before we felt that we could rely upon available dry diets, and these have been continued on the previous régime of 2 parts by weight of proprietary canned dog food to 1 part of cereal biscuit, in fact Spillers' Winalot. The mixture is moistened to a

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semi-solid consistency and given twice daily. The amounts fed are adjusted on a dry-matter content basis to the equivalent of those already indicated for the dry diet. Again, each dog receives the quoted allowance of fresh milk and vitamin tablets. The changes are rung on the proprietary canned food, although for practical convenience the brands that are available in large 7-lb cans are preferred by the operators. This method is employed also in some new experiments in which we have to incorporate the test material in the food and cannot conveniently do so with the dry diet. It should be

TABLE 2. NUTRICON TABLETS (BOB MARTIN LTD.)
(Tablets of vitamins with proteolysed liver)

<i>Formula—each tablet contains:</i>	
Vitamin B group:	
Thiamine (aneurin. HCl; vitamin B ₁)	130 i.u.
Riboflavin (vitamin B ₂)	0.75 mg
Nicotinic acid (niacin)	2.5 mg
Calcium pantothenate	1.0 mg
Pyridoxine (vitamin B ₆)	0.2 mg
Vitamin A	500 i.u.
Vitamin D	250 i.u.
Vitamin E (α-tocopheryl acetate)	0.25 mg
Vitamin B ₁₂ (cyanocobalamin)	1.0 mg
Proteolysed liver	20 mg

Recommended Dosage:

As a nutritional supplement for regular administration:

One or more tablets each day.

For the pregnant and lactating animal:

One or more tablets 3 times a day.

The dose may be increased following illness, or where signs of nutritional deficiency are apparent.

mentioned that for one particular experiment in which it has been felt desirable to incorporate an organophosphorus insecticide in a special dry diet, this has to be made up in Germany and exported to Huntingdon, for the special equipment required did not apparently exist in this country on any premises at which use of the insecticide in these amounts could be approved.

When our numbers of experimental dogs were fewer, the dietary régime comprised cooked fresh meat and wholemeal brown bread, again with milk and vitamin tablets, and with checks on the Ca/P ratio. This was replaced at the week-ends by canned dog food. We were unable to maintain dogs (and even more particularly cats) in good condition for periods of longer than a few weeks on canned food alone. As was discussed at a meeting of the Central Veterinary Society some years ago, a small amount of lactose is—or was—added to certain canned cat foods in order to help maintain bowel function,

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and possibly this additive has been in part responsible for the looseness associated with canned feeding in some laboratories. This was some years ago, and we are aware that previously greyhounds had been raced on one such food and that since then the Canine Health Centre at Newmarket has maintained consistently healthy specimens over at least 3 successive generations on canned food alone. It would be extremely helpful to have full details of this and other controlled investigations, for despite the vast canned dog and cat food industry on both sides of the Atlantic, such information is surprisingly meagre, and at the time of preparation of this paper it was hoped that Hodgman (1965) would be able to add greatly to it. We have seen that he has in fact done so, and we are prepared to investigate the matter further on a larger scale, at any rate in the dog. There remains the need for documented studies on the cat.

The *Which?* (1960) survey on "Petfoods" revealed considerable variation in chemical composition among the relatively small numbers of samples of canned dog and cat foods, and we have confirmed from time to time in our own laboratories that these variations may be within-brand as well as between-brand, at least in respect of certain constituents or criteria of nutritive value, as of course might be anticipated. Some manufacturers have certainly taken steps to correct the thiamine or vitamin B₂ content, which was quite inadequate in some of the samples reported on in the *Which?* survey. It would be most helpful if Mr. Harms could add to the information on stability that was touched upon yesterday.

Fresh water is, of course, available *ad libitum* to our dogs at all times. We have earlier reviewed water requirements and drinking patterns (Worden, Noel and Jolly, 1963).

CATS

In their earlier contributions Scott and her collaborators (Dickinson and Scott, 1956a, b; Scott, Carvalho da Silva and Lloyd Jacob, 1957; Greaves, 1959; Scott, 1960) have dealt in considerable detail with the feeding of experimental cats as well as with available knowledge on nutritional requirements, and in two of the preceding contributions at this Symposium will have brought the latter aspects up to date (Greaves, 1965; Scott, 1965). It is difficult to add to their valuable surveys of the former ones. The stock diet employed at the Royal Free Hospital School of Medicine is simple and inexpensive, and has been found to be successful for growth and reproduction over a period of several years in a colony of 50 to 100 cats. The principal meal, given in the later afternoon at a rate of approximately 100 g per adult cat per day, consists of 2 parts by weight of boiled mash potatoes and 1 part of a protein food, with 1/200 part of dried yeast. The protein food varies, and up to 1957 was described as consisting usually of boiled fish (scrap fish

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obtained from Grimsby) 4 times weekly and cooked minced liver (condemned but not tubercular) 3 times weekly, or canned food (Kit-E-Kat) when these supplies failed. A secondary meal is given in the morning at the rate of 50–80 g per adult cat or growing kittens. For weaning kittens and lactating females this up to 1957 was described as 1 part by volume of Farex precooked fortified biscuit meal, 1 part full cream dried milk powder fortified with vitamin D, with dried yeast added to 1/200 total weight, well mixed to a very stiff paste, since cats dislike sloppy food. Milk is given in limited quantities occasionally. Scott *et al.* (1957) have pointed out that fully adult non-pregnant cats, which are occasional feeders (Hediger, 1950), can manage well on 1 good meal of 150–200 g of food a day, but that it is essential to feed pregnant females twice a day and that lactating females may take 3 meals, owing to the great drain involved in suckling an average litter. New-born kittens are continuous feeders for the first 3 weeks post-partum and gradually become adapted to occasional feeding during weaning. It is hoped that Dr. Scott will indicate in the discussion that her views have not changed, and may be able to tell us also of the adverse effect upon reproduction that, we understand, followed from the enforced absence of liver from the stock diet described.

Scott *et al.* have emphasized that few cats will accept food from the fingers, and that most prefer to feed from small bowls placed at ground level. If food is placed at a higher level it is often dragged out on to the floor of the cage. Some cats eat by scooping up their food with a fore-paw and carrying it to their mouths. All appear to be susceptible to the effects of timing and social effects upon appetite and food intake as described by Katz (1937). At the Royal Free Hospital School of Medicine it has been found that cats appear to “adapt” themselves to their attendant, and that there tends to be a fall in food acceptance—sometimes with accompanying loss of body weight—when a change of attendant occurs as at holiday times, and especially if the new attendant is of the opposite sex to the usual one. “Some moments spent in stroking and speaking to a newly arrived domestic cat will often induce it to take food, once it has gained confidence, whereas if left severely alone it may mope and lose weight.” Dr. Widdowson (1965) has provided a good example of a holiday effect, in the case of her own cat that failed to gain weight over the Christmas week when she was away.

At Huntingdon our regular cat colony is about half the size of that at the Royal Free, and is maintained in outdoor quarters not unlike those described by Weipers (1957). It breeds freely when permitted to do so, and some of the animals in it have remained healthy for a period of over 10 years. Part of the colony consists of animals that have been reared from weaning on a liver-free diet, in order that its members may be utilized for metabolic studies on some of the B vitamins. These animals receive, on a communal basis and *ad libitum*, a mixture of equal parts by weight of cooked fresh minced beef

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and of crumbed wholemeal bread, with the gravy from the cooking added. Fresh milk and fresh water also are available *ad libitum*. The remainder of the holding colony receives, also on a communal basis and *ad libitum*, with milk and fresh water similarly, a mixture of 2 parts by weight of proprietary canned cat food (replacing the cooked rabbit used over the first 5 years) and 1 part of wholemeal bread crumbs. The brand of canned food is deliberately varied so that a succession is fed, but usually the "fish type" is alternated with the "liver type". A check is kept on overall mineral status, and supplements given as required, while a "Tibs" vitamin tablet (see Table 3) is given to each animal daily, on this—although not of course on the liver-free—regimen.

TABLE 3. TIBS TABLETS FOR KITTENS AND CATS

<i>Formula—each tablet contains:</i>	
Thiamine (vitamin B ₁)	0.25 mg
Riboflavine (vitamin B ₂)	0.45 mg
Pyridoxine (vitamin B ₆)	0.12 mg
Niacin	1.50 mg
Calcium pantothenate	0.60 mg
Vitamin A	180 i.u.
Vitamin D	120 i.u.
Vitamin E	0.15 mg
Dried whole liver and liver extract	15 mg
Net weight per tablet 4½ gr.	

We have twice had to undertake subacute toxicity tests in the cat, on the same scale as with dogs, but with each young cat housed in a separate cage. The actual diet was similar to that used for the holding colony, and the daily allowance based upon an initial 50 g dry matter, in 2 feeds, with increases according to appetite.

We have so far only very limited experience of dry complete cat foods, but the writer has seen these used with apparent success in some American laboratories, and hopes that we may be able to make a proper investigation of their suitability in future.

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DISCUSSION

- HARMS: We have carried out a large number of vitamin estimations to test their stability in a variety of canned foods. With the specific diet used by Mr. Hodgman over a period of 3 years, we could find no destruction of vitamin A but there was a 50 per cent loss of vitamin B₁ and B₆ and between 15 and 20 per cent of other B vitamins. With tests carried out on products obtained after storage in shops the indications are that losses are very similar to the above.
- FINDLAY: Can Dr. Worden tell us how he protects food for his outdoor cats from contamination by blowflies?
- WORDEN: We have really had surprisingly little trouble from blowflies. Our outside cattery is protected on three sides by walls or adjacent buildings, and we do, of course, employ a good deal of insecticide.
- BROOYMAN: I have been informed by a dog food manufacturer that a fat content higher than 10 per cent in an expanded meal type dog food would cause an increase in the cost of the packages to such an extent that the product could not be offered at a competitive price. Because of palatability at least part of the fat has to be sprayed on the outside of the pellets and will therefore easily ooze from the pellets under certain climatic conditions if the fat content is high.

APPENDIX V

EFFECT OF RONNEL AFTER CHRONIC FEEDING TO DOGS

by

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TOXICITY OF RONNEL

Ronnel or 0,0-dimethyl-0-(2,4,5-trichlorophenyl) phosphorothioate was subjected to a toxicity study lasting two years in the dog. Throughout the period there were not any overt clinical symptoms that could be attributed to ronnel, and bodyweight did not appear to be affected even at the high level of intake of 10 mg/kg bodyweight/day. There was no evidence of any effect of ronnel upon urinalysis, haematological findings, blood biochemical findings (other than a depression of plasma cholinesterase), organ weights or the histological appearance of the tissues.

Ronnel was without adverse effect upon erythrocyte cholinesterase or brain cholinesterase. Depression of plasma cholinesterase was the only noteworthy effect observed in the study. Depression was significant at the 10mg/kg bodyweight/day dietary level ($P = 0.01$), but was only slight at the intermediate 3mg/kg bodyweight/day level ($P < 0.05$). Dogs in the intermediate group had plasma cholinesterase values which, overall, averaged 82% of pre-treatment controls.

Plasma cholinesterase values for the 1mg/kg bodyweight/day level were indistinguishable from those of controls.

The unequivocal no-effect level of ronnel in dogs is concluded to be 1mg/kg bodyweight/day. However, considering that no other effects were observed in the study and that depression of plasma cholinesterase was generally within a 20% variation from pre-treatment values, it is concluded that 3mg ronnel/kg bodyweight/day produced no significant toxicological effect in dogs.

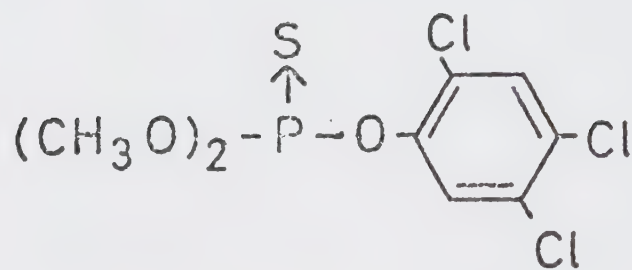


Figure 1 : Structural formula of ronnel or O,O-dimethyl-O-(2,4,5-trichlorophenyl) phosphorothiate

METHODS

32 young pure-bred Pembroke-Shire Corgis were obtained from Appleton & Appleton's kennels during April, 1963, and allocated into 4 groups (each comprising 4 males and 4 females) from May, 1963. Each dog was housed individually. They were treated as described by Worden (1969) except that the basal diet comprised a mixture of canned dog food and a dry dog biscuit (weeks 1 - 85), followed by one of the canned dog foods and Purina Dog Chow (weeks 86 - 104). Daily dietary allowances were initially as described in the case of gusathion, but were increased after 29 weeks to 250g/dog/day and after 40 weeks to 300g/dog/day - the canned meat having a higher moisture content than the dry Dog Chow. One male and one female from each group was sacrificed after 52 weeks.

Ronnel was administered by injection of its solution in arachis oil into a portion of the diet before each meal, using a hypodermic syringe. The concentration of the solution was varied so that each dog received the equivalent of 0.5ml arachis oil/kg bodyweight twice daily, and the same volume of arachis oil itself was incorporated in the diet of the control animals. The dosage rates of ronnel to the three treatment groups were, respectively, 1, 3 and 10mg/kg bodyweight/day, divided between each of the two meals. Over the 2-year period these levels were approximately equivalent to 15, 45 and 150ppm of diet.

Laboratory investigations were carried out prior to the administration of ronnel and at 4, 12, 25, 51, 77 and 103 weeks subsequently. The routine included haematology, urinalysis, serum alkaline phosphatase, serum glutamic oxaloacetic transaminase and the BSP retention test by the method of Lynn (1963). Plasma erythrocyte and terminal brain cholinesterase values were determined by the methods outlined for organophosphorus compounds of Williams *et al.* (1957), Street & Rudd (1968) and of Frawley *et al.* (1952) respectively, but the buffer was changed after 88 weeks from that of Michel (1949) to that of Frawley & Fuyat (1957). Plasma and erythrocyte levels were determined on 4 occasions prior to ronnel administration, and subsequently after 4, 8, 12, 16, 20, 24, 28 weeks and then at calendar month intervals until the end of the study.

INTRODUCTION

Ronnel or 0,0-dimethyl-0-(2,4,5-trichlorophenyl) phosphorothioate is the active ingredient of a systemic insecticide preparation (Trolene) and of a residual insecticide spray (Korlan) and has the structural formula shown in Figure 1.

It is a white powder which, at 99.4% purity, has a melting point of 40.97°C and a vapour pressure of 0.0008mm Hg at 25°C. It is soluble in acetone, carbon tetrachloride, diethyl ether, methylene chloride and toluene, but practically insoluble (0.004% w/w at 25°C) in water (McCollister et al., 1959). Trolene has been shown to be highly effective against warble fly larvae; when administered orally to cattle at a rate of 100mg/kg bodyweight it not only kills the grubs actually in the back but also prevents the encystment of new larvae (McGregor & Bushland, 1956; Roth & Eddy, 1957; Dow Chemical Company, 1958). It has also been reported as having activity against demodectic mange in the dog (Dow Chemical Company, 1958). Korlan is employed against flies in animal buildings and elsewhere and has been found efficacious against ectoparasites of livestock (Hornstein et al., 1958; Knapp et al., 1958; Axtell, 1968; Axtell & Edwards, 1970; Hansens & Anderson, 1970).

Plapp & Casida (1958) showed that ronnel (administered as Trolene) was susceptible to hydrolysis at either the methyl-phosphate or the phenyl-phosphate bond and that both types occurred in rats, houseflies and in a lactating cow. The oxygen analogue, 0,0-dimethyl-0-(2,4,5-trichlorophenyl) phosphate was found to undergo similar hydrolytic cleavage. Of the phosphorus-oxygen-phenyl metabolites appearing in the urine of rats following the oral administration of ronnel, about 40% were 2,4,5-trichlorophenyl phosphorothioic acids and 60% were 2,4,5-trichlorophenyl phosphoric acids. The metabolic pathway of ronnel in the lactating cow was similar to that in the rat, but with a slower rate of detoxification and excretion. The tissue residues of ronnel plus its metabolites were as high 7 days after administration in the cow as one day afterwards in rats.

Radeleff & Woodard (1957) found that a dose level of 400mg/kg bodyweight produced adverse reactions in cattle but was not fatal. McCollister et al., (1959) stated that ronnel had been administered to thousands of cattle at dose levels of from 110 to 150mg/kg bodyweight without the production of harmful effects, except for isolated cases in which other factors were involved, and that dosed at 400mg/kg had not caused adverse effects in sheep or goats, while horses had tolerated 110mg/kg. A dietary level of 0.025% or approximately 14.4mg/kg/day, was well tolerated by pigs over an 18-day period, but double this concentration produced some retardation of bodyweight gain.

McCollister et al., (1959) reported mean LD₅₀ values (g/kg/bodyweight) as follows for eight species: rat, 1.74; guinea pig, 3.14; rabbit, 0.64; mouse, 2.14; dog, > 0.5; duck, > 4.0; fowl, > 5.0; turkey, 0.5. Higher doses always produced vomiting in the dog. When they administered ronnel as a solution in maize oil to the shaved skin of rabbits by the technique of Draize (1955) they recorded 1/12 deaths at 1g/kg bodyweight, 6/12 deaths at 2g/kg and 8/8 deaths at 4g/kg. Eye irritation tests on rabbits and skin irritation and skin sensitization tests on human volunteers produced mild or negative reactions. There was little evidence of potentiation when ronnel was paired with other organophosphorus compounds (malathion, diazionon, systox, methyl parathion, EPN, parathion, phosdrin, gusathion, trithion or schradan) in acute toxicity studies on male rats.

McCollister et al., (1959) reported also the results of dietary studies in dogs and rats. A single dog (a 2-years-old female mongrel) appeared to remain in excellent health when fed approximately 25mg ronnel/kg/day for 11 months and subsequent histopathological investigations were negative. At the end of the study the plasma, erythrocyte and brain cholinesterase levels were adjudged to be 29, 58 and 25%, respectively, of the normal. Four further females (Beagles, initially 8 months of age) were given diets providing approximately 0.3, 1, 3 and 10mg ronnel/kg/day, respectively, over a period of a year, while a fifth animal served as a negative control. Bodyweight gain appeared to be normal in all 5 animals, as did haematological findings, terminal blood urea nitrogen, organ weight

analyses and subsequent histopathological investigations. The overall plasma and erythrocyte cholinesterase levels of the animal receiving the equivalent of 10mg/kg/day were 45 and 64% respectively, those of the control dog, whereas those of the animals receiving lower dosages of ronnel, while exhibiting some fluctuation, did not indicate significant depression.

The rat study was set up with sufficient numbers to permit interim sacrifices from each group at 3, 12 and 18 months, a recovery experiment following withdrawal of ronnel after 3 months, and the maintenance of other animals for 2 years. The dietary concentrations of ronnel provide the approximate equivalents of 0.5, 1.5, 5, and 50mg/kg/day. There was no evidence at any level of a significant effect of ronnel upon mortality, food consumption, bodyweight, haematological findings, organ weight analysis or tumour incidence. At the 50mg/kg level there were indications of liver and kidney damage in both sexes. In the livers there was central lobular, granular degeneration and some necrosis of the parenchymal cells. In the kidneys there was cloudy swelling of the lobular epithelium together with interstitial nephritis. These were present in the animals that had received ronnel for 3 months, as well as for longer periods, but not in the rats that had been allowed to recover. Plasma cholinesterase was affected, particularly in the females, to a greater degree than either erythrocyte or brain cholinesterase, neither of which was depressed at ronnel levels of up to 5mg/kg. Overall plasma cholinesterase activity was reduced to 68% of the control values in males, and to 32% of the control values in females, at the 50mg/kg level.

For the studies reported below, ronnel was received as a white powder from The Dow Chemical Company, Midland, Michigan, and was dissolved in arachis oil.

RESULTS

Clinical observations

Over the whole of the two-year period there were not any overt clinical symptoms that could be associated with the administration of ronnel. During the first few weeks there were a few isolated incidences of vomiting or of refusal to eat at a particular meal, but those occurred in both dosed and negative control animals.

One of the animals in the 3mg/kg group, a male (Dog 131) had recurring episodes of an acute haemolytic anaemia. Nothing abnormal had been noted until week 43, when the dog vomited and ate very little. On examination it was found to be clinically anaemic: all the visible mucous membranes were pale and the tongue was white and almost translucent at the edges. The animal was, however, alert and active, and its rectal temperature was 102.2°F. A specimen of blood taken at this time showed an elevated total white cell count, a marked lowering of Hb (3.4g/100ml), an erythrocyte count of 1.7 millions/cmm and a PCV of 18%. The erythrocyte size was large (MCV, 108cu microns) and the haemoglobin content of each corpuscle was low. (MCHC 19%). The reticulocyte count was 35% of the erythrocyte count.

Dog 131 was treated with an iron preparation and vitamin B₁₂. Throughout the remainder of the experimental period there were episodes when it became clinically anaemic as shown by temporary quietness, occasional weakness and pallor of the visible mucous membranes. Subsequent haematological investigations were made on frequent occasions, and were consistent with the episodic nature of the anaemia. In May, 1965, a blood sample was sent to Dr. A.E. Cronin of the Department of Pathology, University of Cambridge, who found that the direct Coomb's test was strongly positive and who concluded that the evidence was consistent with auto-immune, rather than a drug-induced, allergic haemolytic anaemia. When the animal was examined post mortem, the heart appeared to be enlarged, while the spleen weighed 195g. (1.95% of bodyweight) and measured approximately 25cm x 6cm. Three of the mediastinal lymph nodes in the region of the thymus were enlarged (circa 2cm x 1cm) and

pallid. There was a general pallor of many other tissues, including those of the brain, pancreas and alimentary tract, while the thyroids were small but unequal in size. It was concluded that the isolated findings in this dog were without relevance to ronnel.

Mortality

There were no deaths during the experimental period except for the interim sacrifice of one male and one female from each group after 52 weeks.

Food consumption

This is summarized in Table 1. Analysis of variance was applied to the individual records, and it was found that the variance ratio obtained was below that required to show significance even at the 10% level. All dogs consumed over 90% of the food offered over the whole experimental period.

From the food consumption records, it was calculated that the mean total ronnel intakes of the animals in the three experimental groups kept on experiment for 104 weeks were as follows:-

<u>Dose level</u> (mg/kg/day)	<u>Ronnel intake</u> (g)	<u>Food intake</u> (kg)	<u>Dietary level of ronnel</u> (ppm)
10	54.9	369	150
3	17.0	377	45
1	5.9	381	15

Bodyweight

The weight changes for the first year (32 animals) and for the second year (24 animals) are summarised in Tables 2 and 3, and the scatter of individual gains is indicated in Table 4. Analysis of variance did not reveal any effect from ronnel even at the 10% level of significance.

Urinalysis

After 12 weeks, abnormal numbers of erythrocytes and polymorphonuclear leucocytes were present in the urines of 3 males animals - Dogs 131 and 133, receiving 3mg ronnel/kg/day and Dog 137 (1mg/kg/day) - and were considered to be indicative of a transient urinary infection.

A few erythrocytes were seen in the specimen from a male (Dog 129, received 10mg/kg/day) after 77 weeks, and in those from a female (Dog 128, 10mg/kg/day) and a male (Dog 139, 1mg/kg/day) after 103 weeks, but these were regarded as isolated findings without experimental significance. All other values at all times were considered to be within normal limits.

Haematology and blood chemistry

The results obtained during the pre-dosing investigation appeared to be within normal limits. It was decided that they represented a normal sample for the breed and age, and the grand mean, its standard deviation and the 95% confidence limits are set out in Table 6.

After 4 weeks, a female (Dog 138) receiving 1mg. ronnel/kg/day had raised values as follows:- SAP, 21.6KA units; SGPT, 94 SF units; SGOT, 74 SF units/100 ml. A week later, a further blood specimen was obtained from this animal, by which time the values had returned to within the normal range, viz. SAP, 5.5 KA units; SGPT, 24 SF units; SGOT, 37 SF units/100 ml. A control female (Dog 146) had a somewhat raised SGPT level of 36 KA units/100ml., and the value had fallen to 24 KA units/100ml when re-tested a week later.

After 25 weeks, the control female (Dog 146) had a high total white cell count of 31,300/cmm, of which 24,200/cmm were polymorphonuclear neutrophil leucocytes, and a male (Dog 139) receiving 1mg/kg/day had a plasma urea level of 52mg/100ml.

After 51 weeks, the anaemia in Dog 131, already referred to, was detected. Two of the females receiving 3mg/kg/day (Dogs 132 and 136) had BSP retention values of between 16 and 17%. A male (Dog 139) and a female (Dog 140) receiving 1mg/kg/day, and also a control female (Dog 150)

had plasma urea levels of above 50mg/100ml, but all these had returned to normal when re-tested a week later.

After 77 weeks, a control male (Dog 149) had an ESR of 25 mm/hour while the same two animals that had shown elevated plasma urea levels after 51 weeks did so again - 51 and 58mg/100ml, respectively, in Dogs 139 and 140. On retest a week later, the respective levels had fallen to 47 and 37mg/100ml. Two females (Dog 132, receiving 10mg/kg/day and Dog 144, receiving 1mg/kg/day) had BSP retention values of 19.3% and 18.2% respectively.

After 103 weeks, the same two females (Dogs 132 and 144) had BSP retention values of 12.3% and 11.7% respectively. A control female (Dog 150) had a value of 13.5%, but when retested a week later this had fallen to 8.7%. The 'normal limit' for the BSP test as performed in this study (Lynn, 1963) is difficult to define, although the vast majority of results were below 10% retention.

All other values at all times were regarded as within normal limits, and the group mean results are summarised in Table 7.

Blood cholinesterase levels

The grand mean of the results of the 4 series of pre-dosing investigations was calculated, with results (Δ pH units) as follows:-

Plasma cholinesterase, 0.69 ± 0.077
(range, 0.53 to 0.85)

erythrocyte cholinesterase, 0.20 ± 0.055
(range, 0.08 to 0.31)

The results obtained during the dosing period are summarised in Table 8. Throughout there was not any consistent evidence of an association between the administration of ronnel and erythrocyte cholinesterase values. There was, however, an immediate effect upon plasma cholinesterase in the 10mg group and to a lesser degree, in the 3mg ronnel/kg/day group.

When the experimental group results were compared with those of the control group, using the t-test, the differences may be summarised as follows:-

- (a) The 10mg/kg/day group had levels consistently below those of the control group ($P = 0.01$).
- (b) The 3mg/kg/day group had levels that, on 50% of the occasions they were tested, were below those of the control group ($P = 0.05$). Levels for this test group had an overall average of 82% of the levels for the control group.
- (c) The 1mg/kg/day group, although having levels below those of the control group, did not consistently show any significant difference.

Clearly, 1mg ronnel/kg bodyweight/day was a no-effect level. In the absence of other effects, the slight depression of cholinesterase level at the 3mg/kg bodyweight/day level does not appear to be of practical significance.

Brain cholinesterase levels

The results, obtained from a portion of the left fronto-parietal lobe of the cortex, are listed in Table 9. There was no evidence of any significant effect from ronnel.

Terminal bone marrow counts

Owing to the anaemia that had been observed in Dog 131, as described above, it was felt wise as a precautionary measure to carry out bone marrow counts on the 10mg/kg/day and on the control animals before sacrifice. Sternal samples were therefore taken from all the animals in these two groups (except from the control female, Dog 150) as well as from Dog 131. The results are set out in Table 10. All counts appeared to be within normal limits except for the slight excess of late erythroid elements in Dog 131.

Organ weight analyses

The individual organ weights of the 8 dogs sacrificed after 52 weeks are shown in Table 11, and expressed as a percentage of bodyweight in Table 12.

In all instances, except Dog 131 already referred to, the results were considered to be within normal limits.

Pathological findings

At the interim sacrifice after 52 weeks, no significant macroscopic abnormalities were detected in any of the 8 animals. The female that had received 10mg/kg/day (Dog 122) had three small areas of cyst formation (circa $\frac{1}{4}$ " diameter) on the left uterine horn and a small hard area on the external surface of the left kidney, penetrating for about $\frac{1}{4}$ " into the renal tissue. The male that had received 3mg/kg/day (Dog 129) had an undescended left testicle and a somewhat deep colour of part of the right renal medulla. It had also a small encapsulated area of the left lung, with a papillated appearance, suggestive of innocent tumour formation.

In these 8 animals the changes seen throughout all lungs examined were of a chronic inflammatory nature and, as this condition is indigenous to the dog population employed it was not considered to be of any pathological significance from the point of view of this experiment.

A non-specific granuloma was noted in the submucosa in the oesophagus of one of the high dose dogs and a hypoplasia of the left testis was noted in one of the intermediate dose dogs. A chronic ileitis was noted in one of the low dose dogs. Occasional pituitary cysts were seen throughout all groups. These were considered to be embryological cranio-pharyngeal remnants and as such not to be of any pathological significance. The rest of the tissues examined were within the bounds of normality. There was thus at this interim kill no evidence of alteration in morphology which could be considered to have been induced by ronnel.

At the sacrifice of the remaining 24 animals at 104 weeks, there were again only trivial findings (other than those described for Dog 131), none of which could be attributed to ronnel. There were signs of oestrus, or approaching oestrus in 7 of the females. A male (Dog 123) at the 10mg/kg/day level had a small (circa 0.5cm diameter) dark fawn area on the surface of the right lobe of the liver, penetrating about 1cm into

the tissue beneath and granular in appearance. A similar nodule was present in the spleen, and both were possibly of parasitic origin. There were two firm, pus-filled areas, about 1 x 6 cm, at the periphery of the two lower lobes of the lung, which appeared to be dilated bronchi surrounded by friable tissue and were perhaps areas of bronchiectasis. In a female (Dog 140) at the 1mg/kg/day level the thymus was not found and there were several small (circa 2mm diameter) superficial white spots on the external surface of the kidneys, possibly of parasitic origin.

The histological findings may be summarised as follows:-

Respiratory tract - the histopathological changes were those of a chronic inflammatory reaction, with the exception of a dog in the high dose group and one in the intermediate dose group which showed evidence of an acute bronchopneumonic consolidation, superimposed on the underlying chronic interstitial pneumonitis. These conditions are not uncommon in our dog population, and as such may be disregarded from the point of view of this particular experiment.

Renal tract - this tract was within normal limits, although occasional infiltration of the interstitial spaces with cells of the chronic inflammatory type was seen. This may or may not have been associated with the presence of sub-capsular granulomata which are due to a parasitic infestation with Toxocara canis. In one animal in the low dose groups a small pelvic abscess was noted.

Other anomalies - several histopathological features were seen throughout the animals used in this experiment which bore no relationship to the drug under dose and included a mild encephalitis seen in an animal in the low dose group. Some cystic endometrial hyperplasia was seen in one of the female animals in this group. Pituitary cysts were not uncommon and were seen in many animals throughout the various groups. These are considered to be craniopharyngeal embryological remnants and as such to be without pathological significance.

Gonads - no significant abnormality was detected in either the male or female animals which were sexually mature.

Gastro-intestinal tract - apart from minimal post mortem autolytic change seen in the more superficial layers of the epithelium this system was within the limits of normality.

Endocrine system - within the limits of normality.

Hæmopoietic system - all animals (except for Dog 131) were within the limits of normality.

Liver - the variations in hepatocyte size associated with minimal lymphocytic infiltration around the portal tracts, which, for the most part, were minimal, were considered to be within the limits of normality.

• Central Nervous System - apart from one animal in the low dose group which showed evidence of a mild encephalitis no other abnormality was seen in either the brain, the spinal cord or the peripheral nerves.

There was thus no evidence to suggest any alteration in morphology in the animals used in this experiment which could be attributed to ronnel.

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TABLE 1.

Ronnel study : group mean food consumption (kg/4 week period)
of the 3 dogs per group dosed for 2 years

Dose level of ronnel (mg/kg/day)	Sex	Food Consumption, kg/4 week period			
		0-28 wks.	29-36 wks.	37-84 wks.	85-104 wks.
10	♂	10.77	14.00	15.93	15.86
	♀	10.33	13.98	14.74	16.15
3	♂	11.02	14.00	16.65	16.70
	♀	10.54	14.00	15.41	16.26
1	♂	11.00	13.92	16.59	16.78
	♀	10.97	13.73	16.06	16.03
Controls	♂	11.10	13.93	16.68	16.80
	♀	10.77	13.99	16.36	16.17
Food Allowance		11.2	14.0	16.8	16.80

TABLE 2

Ronnel study : group mean bodyweights and weight gains of dogs at 4-weekly intervals throughout the first 12 months of dosing

Date	Weeks	Group mean bodyweights (g)			Group mean weight gains (g)						
		10mg/ kg/day	3mg/ kg/day	1mg/ kg/day	Controls	10mg/ kg/day	3mg/ kg/day	1mg/ kg/day	Controls		
1963/64 dosed		4♂	4♀	4♂	4♀	4♂	4♀	4♂	4♀	4♂	4♀
24 May	0	6295	6768	7200	6545	7363	6548	6380	6254		
21 June	4	6880	7510	8080	7050	8575	7190	7425	7450	585	742
19 July	8	7193	8245	8333	7615	9210	7555	7878	8188	313	735
16 Aug	12	7405	8328	8820	7820	9178	7635	8093	8523	212	83
12 Sep	16	7238	8408	8858	8310	9128	7688	8108	8693	-167	80
11 Oct	20	7800	8523	9200	8175	8928	7675	8043	8470	562	115
8 Nov	24	7613	8633	8810	8300	9013	8120	8100	8713	-187	110
6 Dec	28	7315	8813	8558	8130	9003	7807	7448	8148	-298	180
3 Jan	32	7238	8040	8338	8230	8575	8050	7418	8063	-77	773
31 Jan	36	7363	8325	8188	8163	8238	8200	7325	7925	125	285
28 Feb	40	8075	8975	8863	8762	8788	8888	7825	8488	712	650
27 Mar	44	8650	9350	9063	8875	9238	9225	8388	8975	575	375
24 Apr	48	8875	9475	9250	9025	9250	9238	8250	9175	225	125
15 May	51	8713	9163	9650	8825	9643	9400	8500	9563	-162	-312
Total											
gain 0-51		2418.2395 2450.2270 2280.2852 2020 3309									

		2407 2360 2566 2665									

TABLE 3

Ronnel study : group mean bodyweights and weight gains of dogs at 4 weekly intervals throughout the second 12 months of dosing

Date	Weeks	Group mean bodyweights (g)				Group mean weight gain (g)											
		10 mg/ kg/day	3 ♀	3 ♂	1 mg/ kg/day	Controls	10 mg/ kg/day	3 ♀	3 ♂	3 mg/ kg/day	3 ♀	3 ♂	1 mg/ kg/day	Controls			
1964/65 dosed																	
		3 ♂	3 ♀	3 ♂	3 ♀	3 ♂	3 ♀	3 ♂	3 ♀	3 ♂	3 ♀	3 ♂	3 ♀	3 ♂	3 ♀		
22 May	52	8900	8717	9217	9333	9450	9667	8767	9817	366	216	-167	67	366	216		
19 June	56	9266	8933	9050	9400	9816	9883	8783	10133	-66	-167	50	-34	50	50		
17 July	60	9200	8766	9100	9366	9866	9933	8766	10000	-17	194	-149	317	-50	-17		
14 Aug	64	9183	8960	8951	9683	9816	9916	8983	10266	250	106	265	233	-166	250		
11 Sep	68	9433	9066	9216	9916	9650	10166	9100	10300	17	284	-66	117	233	117		
9 Oct	72	9450	9350	9150	10033	9883	10283	9233	10650	-50	-217	-150	-203	-417	-67		
6 Nov	76	9400	9133	9000	9830	9466	10216	8850	10480	100	-17	-67	86	-450	184		
4 Dec	80	9500	9116	8933	9916	9016	10400	8916	10533	50	100	-83	267	534	116		
1 Jan	84	9550	9216	8850	10183	9550	10516	8866	10616	383	300	583	100	333	134		
29 Jan	88	9933	9516	9433	10283	9883	10650	9233	11050	784	284	400	434	750	750		
26 Feb	92	10717	9800	9833	10717	10633	11400	10017	11650	-367	-150	-17	-134	-200	-200		
26 Mar	96	10350	9650	9816	10583	10433	11200	9816	11583	-17	33	434	367	-117	300		
23 Apr	100	10333	9683	10250	10950	10316	11500	10333	11766	167	267	100	50	284	-33		
14 May	103	10500	9950	10350	11000	10700	11467	10083	11717								

Total		52-103				1600				1233				1667			
gain																	

TABLE 4

Ronnel study : bodyweight changes of individuals
after 1 year and 2 years dosing

Dose level of ronnel (mg/kg/day)	Dog No. Sex	Bodyweights (g)			Weight gain (g)	
		Week 0	Week 51	Week 103	Over 1 year	Over 2 years
10	121♂	7210	8550		1340	
	123♂	6390	10650	13000	4260	6610
	125♂	5770	8450	10350	2680	4580
	127♂	5810	7200	8150	1390	2340
	122♀	8040	11300		3260	
	124♀	6620	8950	10850	2330	4230
	126♀	6220	8400	9300	2180	3080
	128♀	6190	8000	9700	1810	3510
3	129♂	8690	11050		2360	
	131♂	5760	9750	10200	3990	4440
	133♂	6450	8850	10500	2400	4050
	135♂	7900	8950	10350	1050	2450
	130♀	6860	7750		890	
	132♀	6530	11500	13600	4970	7070
	134♀	6800	7600	9250	800	2450
	136♀	5990	8450	10150	2460	4160
1	137♂	8630	10250		1620	
	139♂	7800	8250	9500	450	1700
	141♂	6640	11050	12200	4410	5560
	143♂	6380	9100	10400	2720	4020
	138♀	7220	8750		1530	
	140♀	6350	9550	10650	3200	4300
	142♀	5750	8850	10650	3100	4900
	144♀	6870	10450	13100	3580	6230
Controls	145♂	7000	7950		950	
	147♂	6520	9550	10100	3030	3580
	149♂	6340	9150	11450	2810	5110
	151♂	5660	7350	8700	1690	3040
	146♀	6040	9050		3010	
	148♀	6250	9500	10350	3250	4100
	150♀	7100	9300	12000	2200	4900
	152♀	5630	10400	12800	4770	7170

TABLE 5

Mean and standard deviation of haematology and blood chemical values of 32 young Pembroke-shire Corgis prior to experimental dosing

Investigation	Units	No of specimens	MEAN	Standard deviation	95% Confidence limits	
Hb	g/100ml	32	13.8	0.89	12.0	- 15.6
PCV	% red cells	32	44 .	4.16	34.9	- 51.9
RBC	$10^6/\text{cmm}$	32	5.55	0.38	4.78	- 6.32
MCHC	%	30	31	1.94	27	- 35
MCV	cubic	30	79	5.72	67	- 91
Total WBC	$10^3/\text{cmm}$	30	15.5	5.38	4.53	- 26.45
Neutrophils	cmm	30	9.7	4.01	1.48	- 17.82
Lymphocytes	$10^3/\text{cmm}$	30	4.7	1.85	0.94	- 8.48
Urea	mg/100ml	32	17	7.74	1.3	- 32.8
SAP	KA	32	11.8	3.34	4.7	- 18.8
SGPT	SF	32	9.4	2.98	3.3	- 15.5
SGOT	SF	32	36.3	6.78	22.5	- 50.1

TABLE 6

Ronnel study : group mean haematology values of dogs

Dose level (mg/kg/day)	Weeks dosed	PCV %	Hb (g/100ml)	RBC (10^6 / cmm)	MCHC %	MCV μ	WBC Total	N	L
Control	4	45	14.9	5.67	33	79	16.0	8.6	6.4
	12	52	18.2	6.54	35	80	18.1	11.2	6.2
	26	50	17.0	6.31	32	83	15.0	10.0	4.4
	52	51	15.0	5.85	30	86	13.3	9.5	2.5
	77	48	15.1	5.81	31	83	13.4	7.4	5.0
	103	53	17.3	6.36	33	83	9.7	5.5	3.4
1	4	45	14.9	5.75	33	78	13.3	6.4	6.2
	12	50	17.9	6.31	36	79	17.3	10.0	6.2
	26	50	15.3	6.29	30	80	14.7	10.2	4.0
	52	49	14.8	5.84	30	84	11.1	8.4	1.3
	77	49	15.2	5.88	31	83	12.7	7.5	3.9
	103	52	17.0	6.18	32	84	10.3	6.1	3.1
3	4	45	14.8	5.76	33	78	14.0	7.8	5.2
	12	50	17.7	6.14	36	81	16.0	8.9	5.9
	26	50	15.0	6.04	30	82	11.4	8.0	2.8
	52	44	13.2	5.04	29	91	14.0	10.8	2.1
	77	45	14.1	5.57	31	81	14.4	9.4	4.3
	103	49	15.5	5.58	31	89	11.3	7.5	3.1
10	4	46	14.9	5.70	32	81	13.9	6.7	5.8
	12	51	17.3	6.25	34	82	20.8	13.8	5.6
	26	47	15.7	5.78	33	81	14.5	10.1	3.7
	52	49	14.5	5.73	30	85	16.3	12.0	2.7
	77	48	14.9	6.03	31	79	12.4	7.5	4.2
	103	51	16.6	6.08	32	84	12.2	7.2	3.8

TABLE 7

Ronnel study : group mean blood chemistry and BSP values of dogs

Dose level (mg/kg/day)	Weeks dosed	Urea (mg/100ml)	SAP (KA units)	SGPT (SF units)	SGOT (SF units)	BSP (%)
Control	4	23	7.9	12	32	5.4
	12	23	10.1	10	32	-
	26	28	4.8	11	22	5.6
	52	42	6.8	17	29	5.2
	77	32	4.0	13	23	-
	103	26	3.8	22	17	7.0
1	4	23	8.8	9	40	4.2
	12	27	7.7	6	29	-
	26	31	5.2	10	26	5.8
	52	44	5.8	8	28	5.3
	77	37	3.4	15	26	-
	103	31	3.3	27	24	6.9
3	4	23	8.1	13	30	3.8
	12	26	6.6	10	34	-
	26	28	4.1	11	33	5.5
	52	31	5.1	7	28	7.6
	77	30	3.5	15	25	-
	103	25	3.2	21	27	6.0
10	4	20	5.2	11	34	4.8
	12	28	8.1	9	26	-
	26	31	6.6	11	38	6.4
	52	35	6.1	6	22	6.2
	77	30	4.3	15	27	-
	103	29	4.0	23	25	4.7

TABLE 8

Ronnel study : analysis of group mean cholinesterase levels of dogs

Date 1963/4/ 5.	Weeks dosed	No of degrees of freedom	PLASMA				Variance Sig.				RBC			
			Group mean levels				Ratio (F)				Group mean levels			
			10mg/ kg/day	3mg/ kg/day	1mg/ kg/day	Con- trols	10mg/ kg/day	3mg/ kg/day	1mg/ kg/day	Con- trols	10mg/ kg/day	3mg/ kg/day	1mg/ kg/day	Con- trols
0	0	3+28	.81	.78	.70	.72	1.35				.21	.19	.19	.20
0	0	"	.76	.79	.69	.81	2.11				.16	.19	.20	.18
0	0	"	.66	.64	.62	.62	0.38				.22	.25		
0	0	"	.69	.64	.53	.63	3.11	*			.19	.20	.19	.20
18 Jun	4	1	.67 ⁺⁺	.84	.97	1.06	7.68	**			.17	.28	.42	.41
12 Jul	8	"	.94 ⁺	1.23	1.13	1.13	15.62	**			.27	.35	.26	.30
19 Aug	12	"	.59 ⁺⁺	.75	.64	.71	6.88	**			.15	.21	.14	.16
17 Sep	16	"	.59 ⁺⁺	.64 ⁺⁺	.71 ⁺⁺	.77	8.43	**			.16	.15	.18	.16
15 Oct	20	"	.53 ⁺⁺	.58 ⁺⁺	.65	.72	10.24	**			.14	.19	.14	.13
12 Nov	24	"	.51 ⁺⁺	.61	.61	.64	2.08				.11	.13	.13	.11
10 Dec	28	"	.53 ⁺⁺	.55 ⁺⁺	.64 ⁺	.73	7.94	**			.15	.12	.13	.13
15 Jan	34	"	.46 ⁺⁺	.56	.51 ⁺	.56	3.60	*			.25	.22	.16	.16
14 Feb	38	"	.42 ⁺⁺	.48 ⁺	.50	.57	6.07	**			.19	.18	.16	.16
11 Mar	42	"	.40 ⁺⁺	.54	.55	.55	6.64	**			.16	.16	.11	.12
7 Apr	46	"	.59 ⁺	.64	.71	.70	3.76	*			.16	.09	.11	.10
11 May	50	"	.51 ⁺⁺	.61	.65	.67	3.58	*			.19	.16	.13	.14
15 Jun	56	3+20	.49	.51	.52	.63	5.06	**			.21	.22	.16	.13
14 Jul	60	"	.39 ⁺⁺	.48 ⁺⁺	.48 ⁺⁺	.67	8.82	**			.17	.17	.16	.16
17 Aug	65	"	.51 ⁺	.56	.47 ⁺⁺	.62	0.80				.14	.16	.15	.17
9 Sep	68	"	.41 ⁺⁺	.51 ⁺⁺	.65	.70	11.02	**			.20	.25	.20	.21
6 Oct	72	"	.59	.60	.66	.68	2.01				.16	.15	.18	.17
5 Nov	76	"	.38 ⁺⁺	.46 ⁺⁺	.59	.71	7.58	**			.14	.15	.18	.20
4 Dec	80	"	.34 ⁺⁺	.41 ⁺⁺	.60 ⁺	.81	9.59	**			.13	.12	.13	.14
29 Dec	84	"	.45 ⁺⁺	.56 ⁺	.64	.81	3.63	*			.12	.14	.12	.15
27 Jan	88	"	.43 ⁺⁺	.50 ⁺	.60	.67	3.49	*			.12	.11	.10	.12

TABLE 8
(continued)

Date	Weeks dosed	No of degree of freedom	PLASMA				RBC					
			Group mean levels				Variance Sig.					
			10mg/ kg/day	3mg/ kg/day	1mg/ kg/day	Con- trols	Ratio (F)	5% 1%	10mg/ kg/day	3mg/ kg/day	1mg/ kg/day	Con- trols
4 Dec	80	FM	.48 ⁺⁺	.59 ⁺⁺	.80 ⁺	1.13	8.82	**	.75	.89	.82	.90
29 Dec	84	FM	.65	.78	.77	.93	1.32		.58	.71	.69	.67
27 Jan	88	FM	.54 ⁺⁺	.62 ⁺	.69	.85	3.06		.69	.92	.72	.85
23 Feb	92	F	.53 ⁺	.62	.62	.71	2.34	*	.72	.77	.69	.75
24 Mar	96	F	.44 ⁺⁺	.52 ⁺	.56	.71	3.45	*	.62	.73	.65	.68
22 Apr	100	F	.49	.55	.65	.65	3.34	*	.73	.86	.72	.70
11 May	104	F	.65 ⁺	.64 ⁺	.69	.79	2.50		.65	.76	.63	.69

+ Significantly different from control value (at 5% level)
++ " " " " (at 1% level)

F Using Frawley buffer

FM Using Frawley and Michel buffer (duplicate results)

TABLE 9

Ronnel study : brain cholinesterase levels of dogs
after 2 years of dosing

Dog No. Sex	Dose level Ronnel mg/kg/day	pH/hr/gm wet weight	Dog No. Sex	Dose level Ronnel mg/kg/day	pH/hr/gm wet weight
123♂	10	10.5	139♂	1	9.5
125♂		18.8	141♂		19.4
127♂		9.3	143♂		13.0
124♀		13.5	140♀		7.3
126♀		18.7	142♀		14.2
128♀		7.2	144♀		11.7
Mean		13.0	Mean		12.5
131♂	3	12.2	147♂	Controls	9.6
133♂		2.7	149♂		11.5
135♂		14.4	151♂		19.1
132♀		17.1	148♀		12.0
134♀		2.4	150♀		10.5
136♀		9.4	152♀		6.7
Mean		9.7	Mean		11.6

TABLE 10

Ronnell study : bone marrow counts (% total count) of dogs at termination

Dose mg/kg/day	Dosed												Controls
	10 mg/kg/day												
Dog No./ Sex	123♂	125♂	127♂	124♀	126♀	128♀	131♂	147♂	149♂	151♂	148♀	150♀	152♀
Total count x10 ³ cells/cmm	84.0	145.0	125.0	225.0	219.0	156.0	56.0	161.0	209.0	84.0	96.0	n.s.	156.0
Total Myeloid	73.0	55.0	62.5	49.5	50.0	63.5	50.5	59.0	69.0	65.0	62.5		52.0
" Lymphoid	10.0	11.5	10.0	8.5	7.5	6.5	5.0	7.0	5.0	8.0	16.0		4.5
" Erythroid	17.0	33.0	26.5	40.5	41.0	29.0	44.0	33.5	26.0	26.5	21.5		42.0
" Megakaryocytes	-	0.5	-	-	-	-	-	-	-	-	-		-
M/E Ratio:-1	4.3	1.7	2.4	1.2	1.2	2.2	1.1	1.8	2.7	2.5	2.9		1.2
Segmented Polys.	6.0	2.5	5.0	1.5	1.0	5.5	2.5	4.5	-	3.5	4.0		1.0
Non-segmented Polys.	57.0	43.5	50.0	42.5	41.0	48.0	43.0	45.5	59.5	56.5	57.0		40.5
Metamyelocytes	4.0	2.0	3.0	4.0	1.5	1.5	2.5	3.0	3.0	2.0	-		3.5
Myelocytes	1.0	2.0	2.0	1.0	2.0	2.5	-	1.5	2.0	2.0	1.0		3.5
Promyelocytes	1.0	1.5	-	-	0.5	1.0	1.0	0.5	1.0	0.5	-		1.5
Myeloblasts	-	0.5	-	-	0.5	0.5	-	1.0	1.0	0.5	-		-
Eosinophils	3.0	3.0	1.5	0.5	2.5	2.0	1.0	2.0	0.5	-	0.5		0.5
Eosinophils													
Metamyelocytes	1.0	0.5		1.0	2.5	0.5	0.5	1.5					1.5
Eosinophils Myelocytes		0.5					0.5						
Basophils													
Basophils Myelocytes													

n.s. No sample obtained

TABLE 10
(continued)

Dose mg/kg/day	Dosed										Controls		
	10 mg/kg/day												
Dog No./Sex	123♂	125♂	127♂	124♀	126♀	128♀	131♀	147♂	149♂	151♂	148♀	150♀	152♀
Lymphocytes	9.0	8.5	8.5	7.5	5.0	4.0	4.0	6.5	4.5	6.0	11.0		4.0
Polymphocytes	1.0	1.0	1.0		1.0	0.5	1.0			1.5	1.5		0.5
Lymphoblasts				0.5						0.5	-		
Monocytes		1.0			0.5	0.5	0.5				1.0		
Promonocytes		0.5				0.5					0.5		
Monoblasts													
Plasma cells		0.5	0.5	0.5	1.0	1.0		0.5	0.5		2.0		
Proplasma cells													
Normoblasts -													
Late	14.0	17.0	13.5	25.5	21.5	13.0	31.0	21.0	19.0	18.0	14.5		29.0
Inter	2.0	15.0	12.0	14.5	18.0	15.0	12.5	11.5	5.5	7.5	6.5		11.5
Early	1.0	1.0	1.0	0.5	1.5	1.0	0.5	1.0	1.5	1.0	0.5		1.5
Megaloblast													
Proerythroblasts													
Mitotic cells		1.0	0.5			0.5							0.5
RBC			1.0	1.5	0.5			0.5	0.5				1.0

TABLE 11

Ronnel study : organ weights of dogs in grammes

Dog No./Sex	Body wt	Brain	Pituitary	Heart	Lungs	Liver	Spleen	Pan-creas	Thy-mus	Prostate	Kidneys		Thyroid		Adrenal		Gonad	
											L	R	L	R	L	R	L	R
<u>Dose level 10 mg Ronnel/kg/day</u>																		
121♂	8100	90.0	.060	66.6	81.0	207.0	16.5	18.5	2.5	5.5	17.5	17.5	0.22	0.20	0.45	0.42	12.0	13.
122♂	11000	87.5	.080	83.0	93.5	261.5	17.0	24.0	10.0	10.0	22.5	23.0	0.30	0.40	0.50	0.60	1.5	1.
<u>Dose level 3 mg Ronnel/kg/day</u>																		
129♂	10900	83.7	.050	100.7	89.5	241.1	23.5	30.3	11.8	4.5	20.8	22.9	0.35	0.38	0.51	0.57	12.5	3.
130♂	7500	69.5	.050	58.0	65.5	172.0	17.0	24.0	1.5	15.0	15.5	15.2	0.23	0.27	0.55	0.52	0.85	0.7
<u>Dose level 1 mg Ronnel/kg/day</u>																		
137♂	9600	107.5	.060	87.5	95.0	233.0	12.5	21.0	4.0	10.5	21.5	21.0	0.34	0.33	0.45	0.50	16.5	17.
138♂	8200	97.0	.050	77.0	75.5	206.5	12.5	22.5	6.5	2.5	20.0	19.0	0.30	0.30	0.67	0.67	0.40	0.4
<u>Controls</u>																		
145♂	7850	89.5	.048	70.0	61.0	214.5	10.0	17.0	3.0	5.0	20.0	21.7	0.30	0.27	0.42	0.43	10.5	11.
146♂	8300	86.0	.050	94.0	88.0	204.0	14.8	26.0	12.2	5.5	19.0	20.0	0.25	0.24	0.42	0.48	0.25	0.2

TABLE 12

Ronnell study : organ weights of dogs as a percentage of bodyweight

Dog No./ Sex	Body wt.	Brain	Pituitary	Heart	Lungs	Liver	Spleen	Pan- creas	Thy- mus	Prostate/ uterus	Kid- neys	Thy- roids	Adre- nals	Gonads
<u>Dose level 10 mg Ronnel/kg/day</u>														
121♂	100	1.11	.00074	0.82	1.00	2.56	0.20	0.23	.031	.068	0.43	.0052	.0107	.309
122♀	100	0.70	.00073	0.75	0.85	2.38	0.15	0.22	.091	.091	0.41	.0064	.0100	.027
<u>Dose level 3 mg Ronnel/kg/day</u>														
129♂	100	0.77	.00046	0.92	0.82	2.21	0.22	0.28	.108	.041	0.40	.0067	.0099	.146
130♀	100	0.93	.00067	0.77	0.87	2.29	0.23	0.32	.020	.200	0.41	.0067	.0143	.021
<u>Dose level 1 mg Ronnel/kg/day</u>														
137♂	100	1.12	.00063	0.91	0.99	2.43	0.13	0.22	.042	.109	0.44	.0070	.0099	.354
138♀	100	1.18	.00061	0.94	0.92	2.52	0.15	0.27	.079	.031	0.48	.0073	.0163	.011
<u>Controls</u>														
145♂	100	1.14	.00061	0.89	0.78	2.74	0.13	0.22	.038	.064	0.53	.0073	.0109	.274
146♀	100	0.98	.00057	1.07	1.00	2.32	0.17	0.30	.139	.067	0.44	.0059	.0102	.006

